UNIVERSITY OF CALIFORNIA
Santa Barbara

Department of Chemistry and Biochemistry

# Total Synthesis of Isotopically-Labeled Cylindrospermopsin Cyanotoxins

A thesis submitted in partial satisfaction of the
requirements for the degree Master of Science in Chemistry

by

Joanna Lihua Chen

Committee in charge:
Professor Armen Zakarian, Committee Chair
Professor Liming Zhang
Professor Javier Read de Alaniz
Professor Trevor Hayton

January 2018

The thesis of Joanna Lihua Chen is approved.

_____

Professor Liming Zhang

_____

Professor Javier Read de Alaniz

_____

Professor Trevor Hayton

_____

Professor Armen Zakarian, Committee Chair

January 2018

Total Synthesis of Isotopically-Labeled Cylindrospermopsin Cyanotoxins



by

Joanna Lihua Chen

ACKNOWLEDGEMENTS

I would like to thank Professor Armen Zakarian for his direction and advice. His insights have been valuable to both my projects and to my understanding of chemistry. I would also like to thank Professor Liming Zhang, Professor Javier Read de Alaniz, and Professor Trevor Hayton for their service as members on my committee.

I thank the Zakarian group members, both past and present for their guidance, discussions, and support. They have been some of the best people and mentors I have encountered in this journey. I wish them the best in their future endeavors.

I am truly thankful to my undergraduate advisor, Professor André K. Isaacs, my journey through graduate school would not have even begun without his direction and encouragement.

Finally, I would like to acknowledge my family for their kindness, understanding, and encouragement. Without their love and support, none of this would be possible.

Total Synthesis of Isotopically-Labeled Cylindrospermopsin Cyanotoxins

Joanna Lihua Chen

Thesis Advisor: Professor Armen Zakarian

## ABSTRACT

Cyanobacteria, also known as blue-green algae, are prokaryotic organisms that inhabit freshwater and brackish lakes. They produce toxic secondary metabolites known as cyanotoxins, the increased occurrence of which is believed to be a result of eutrophication. Among them, cylindrospermopsin and its derivatives have become the subject of intense interest from food safety agencies and the scientific community due to their rapidly expanding global presence. Toxins produced by cyanobacterial blooms pose a threat to the aquatic ecosystem and its biodiversity. For these reasons, various analytical methods have been developed for the quantitative monitoring of cylindrospermopsin levels in fresh water and fish. However, in all cases, sufficient accuracy, reproducibility, and standard curve linearity have only been demonstrated for narrow concentration ranges or for relatively high limits of quantification. Isotopically-labeled compounds serve as primary analytical standards in isotope-dilution mass spectrometry (IDSM) - an analytical method of highest metrological quality. A common limiting factor in application of these compounds is access to pure samples with isotope placement at non-exchangeable positions to avoid a loss of label. Here, we demonstrate this concept by a concise and scalable total synthesis of the complex cyanobacterial alkaloids cylindrospermopsin, 7-*epi*-cylindrospermopsin, and 7-

deoxycylindrospermopsin from [$^{15}$N]-ammonium chloride, in which all nitrogen atoms are in the form of $^{15}$N isotope with greater than 99.5% isotope incorporation.

TABLE OF CONTENTS

## List of Schemes

## List of Tables

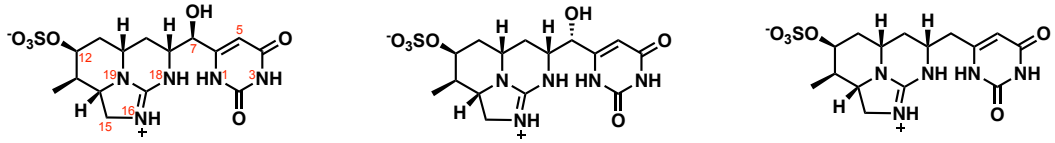
## Table of Figures

## Chapter 1: Introduction and Background

### Cylindrospermopsin and related cyanobacterial alkaloids

Cylindrospermopsin **1** is a toxin produced by harmful algae blooms of cyanobacteria found at the surface of fresh and brackish water (Figure **1**). Cylindrospermopsin was discovered in 1979 when an outbreak in Palm Island in Queensland, Australia led to the hospitalization of 138 children and 10 young adults with anorexia, malaise, vomiting, and enlarged kidneys after drinking from the town's common water supply, Solomon Dam.[1] The reservoir had been experiencing a heavy algal bloom leading up to the "Palm Island mystery disease", after the accident, the potential toxicity of cyanobacterial species was investigated by Hawkins *et al*.[2] In 1992, cylindrospermopsin was isolated from *C. raciborskii*. The toxin was purified, characterized and named.[3] Since its discovery, cylindrospermopsin was also found produced by a variety of cyanobacteria genera including: *Cylindrospermopsis raciborskii* (*C. raciborskii*), *Aphanizomenon ovalisporum*, *Umezakia natans*, *Aphanizomenon flos-aquae*, *Aphanizomenon gracile*, *Anabaena bergii*, *Rhaphidiopsis mediterranea*, *Lyngbya wollei*, *Anabaena planctonica*, *Anabaena lapponica* and *Rhaphidiopsis curvata*.[4]

**Figure 1.** Chemical structures for cyanobacterial toxins cylindrospermopsin, 7-*epi*-cylindrospermopsin and 7-deoxycylindrospermopsin.



cylindrospermopsin, 1 | 7-*epi*-cylindrospermopsin, 2 | 7-deoxycylindrospermopsin, 3

### Chemical structures of cylindrospermopsin alkaloids

To date, three naturally occurring cylindrospermopsin variants are known: cylindrospermopsin, 7-*epi*-cylindrospermopsin **2**, and 7-deoxycylindrospermopsin **3** (Figure **1**). The cylindrospermopsin alkaloids are zwitterionic containing a tricyclic guanidine core and an uracil moiety. Cylindrospermopsin and 7-*epi*-cyclindrospermopsin contain six stereocenters, differing only in the stereoconfiguration of the hydroxyl group at C-7, with cylindrospermopsin having *R*-configuration and 7-*epi*-cyclindrospermopsin having *S*-configuration. Early literature had reversed the stereochemical assignment between cylindrospermopsin and its epimer. In 2001, Weinreb reported the asymmetric total synthesis of 7-*epi*-cylindrospermopsin and corrected for the assignment of the hydroxyl group at the C-7 position.[5]

**Toxicity**

Cylindrospermopsin, classified as a cytotoxin, is harmful to several organs including the liver, kidneys, intestine and adrenal glands. The toxin has been shown to be a potent inhibitor of several protein synthesis pathways.[6-9] Cylindrospermopsin has also shown *in vitro* mutagenic, carcinogenetic and genotoxic activity.[10-13] 7-*epi*-cylindrospermopsin show similar toxicity in mouse bioassay.[14] While 7-deoxycylindrospermopsin has shown to be generally nontoxic in mouse bioassay by intraperitoneal injection, it exhibits similar protein synthesis and cell proliferation *in vitro* to that of cylindrospermopsin.[15-16] The cyanotoxins can be introduced into human body by consumption of contaminated drinking water, fish, and shellfish from bodies of water experiencing cyanobacterial blooms. Since its discovery, cylindrospermopsin has emerged as one of the most important toxins in freshwater.

Cylindrospermopsin has become the subject of intense interest from food safety agencies due to its rapidly expanding global presence. Toxins produced by cyanobacterial blooms pose a threat to aquatic ecosystems and their biodiversity. Cylindrospermopsin has been shown to be toxic to many species of plants, bacteria, protozoa, invertebrates, and vertebrates, including humans.[1] The event in Palm Island, Australia makes evident that drinking or consuming water contaminated with cylindrospermopsin causes serious human illness. Since its occurrence in Australia, cylindrospermopsin have also been detected in New Zealand, Europe, Africa, Asia and North America.[1] Within the United States, harmful *C. raciborskii* blooms have been detected in Indiana, Louisiana, Ohio, Florida, Oregon, Michigan, New York and the Great Lakes.[17-20] Because cylindrospermopsin can enter the food chain through municipal water supplies or by contamination of fish and seafood, various analytical methods have been developed for the quantitative monitoring of cylindrospermopsin levels in freshwater and fish tissues.

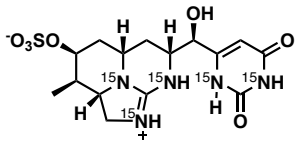
**Detection by Mass-Spectrometry**

Methods including bioassays, immunoassays, and various chromatographic techniques have been developed for the detection and the quantitative measurement of cylindrospermopsin levels in fish and water samples.[1] Among these methods, high performance liquid chromatography-mass spectrometry (HPLC-MS) was deemed the most effective due to high selectivity and unsurpassed sensitivity. However, in all cases, sufficient accuracy, reproducibility, and standard curve linearity have only been demonstrated for narrow ranges of concentrations. It has been well documented that inclusion of an internal standard improves the precision of quantitative analysis and corrects for the nonlinearity in

the standard curve.[21] By inclusion of an internal standard, area ratios will be plotted against the known concentration of the internal standard rather than the absolute intensity of the often-compromised MS signal. On this note, isotope-dilution mass spectroscopy (IDMS) has emerged as one of the most powerful and precise analytical methods,[22] therefore, a high-quality internal standard for the quantification of cylindrospermopsin by IDMS is desirable. Moreover, an isotope of cylindrospermopsin with a mass difference of at least 5 atomic mass units relative to the most abundant natural isotope is pursued.

An ideal solution for the reliable analysis of cylindrospermopsin in water supplies and freshwater seafood is dependent on the availability to access the isotopically-labeled natural product such as [$^{15}N^{1}$, $^{15}N^{3}$, $^{15}N^{16}$, $^{15}N^{18}$, $^{15}N^{19}$]-cylindrospermopsin, ([$^{15}N_5$]-cylindrospermopsin **4** (Figure **2**). However, although cylindrospermopsin itself is not available commercially on a consistent basis, the toxin can be isolated in small quantities from the cultures of *C. raciborskii*.[23-24] Attempts to use a similar approach to produce labeled material met with significant challenges, as cultures supplemented with $^{15}NH_4Cl$ required two years to produce low-quality material not suitable as the analytical standard. The current approach is unreliable and requires an exceedingly long time to permit optimization.[25] In light of this background, total synthesis of isotopically-labeled cylindrospermopsin is an appealing alternative as a scalable and consistent source of pure material. Moreover, the total synthesis of cylindrospermopsin can potentially be readily adopted to provide isotopically-labeled material for the detection of the natural toxin using sensitive and accurate IDMS methods. [$^{15}N_5$]-cylindrospermopsin **4** is chosen as a synthetic target in our group.

**Figure 2.** Chemical structure of isotopically-labled cylindrospermopsin ([$^{15}N_5$]-cylindrospermopsin).



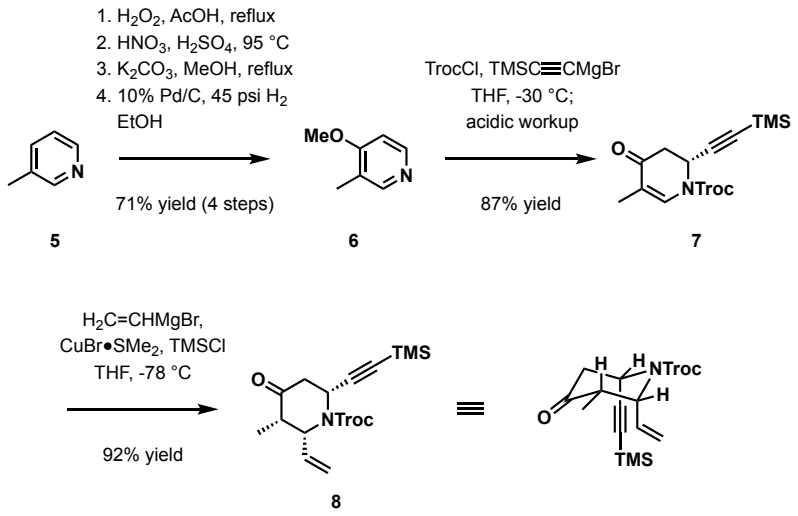

[$^{15}N_5$]-cylindrospermopsin, 4

**Synthetic approaches to 7-*epi*-cylindrospermopsin**

To date, four total syntheses of cylindrospermopsins have been described, all of which targeted 7-*epi*-cylindrospermopsin **2**, each expanding the knowledge of chemistry and biology of the cylindrospermopsin alkaloids.

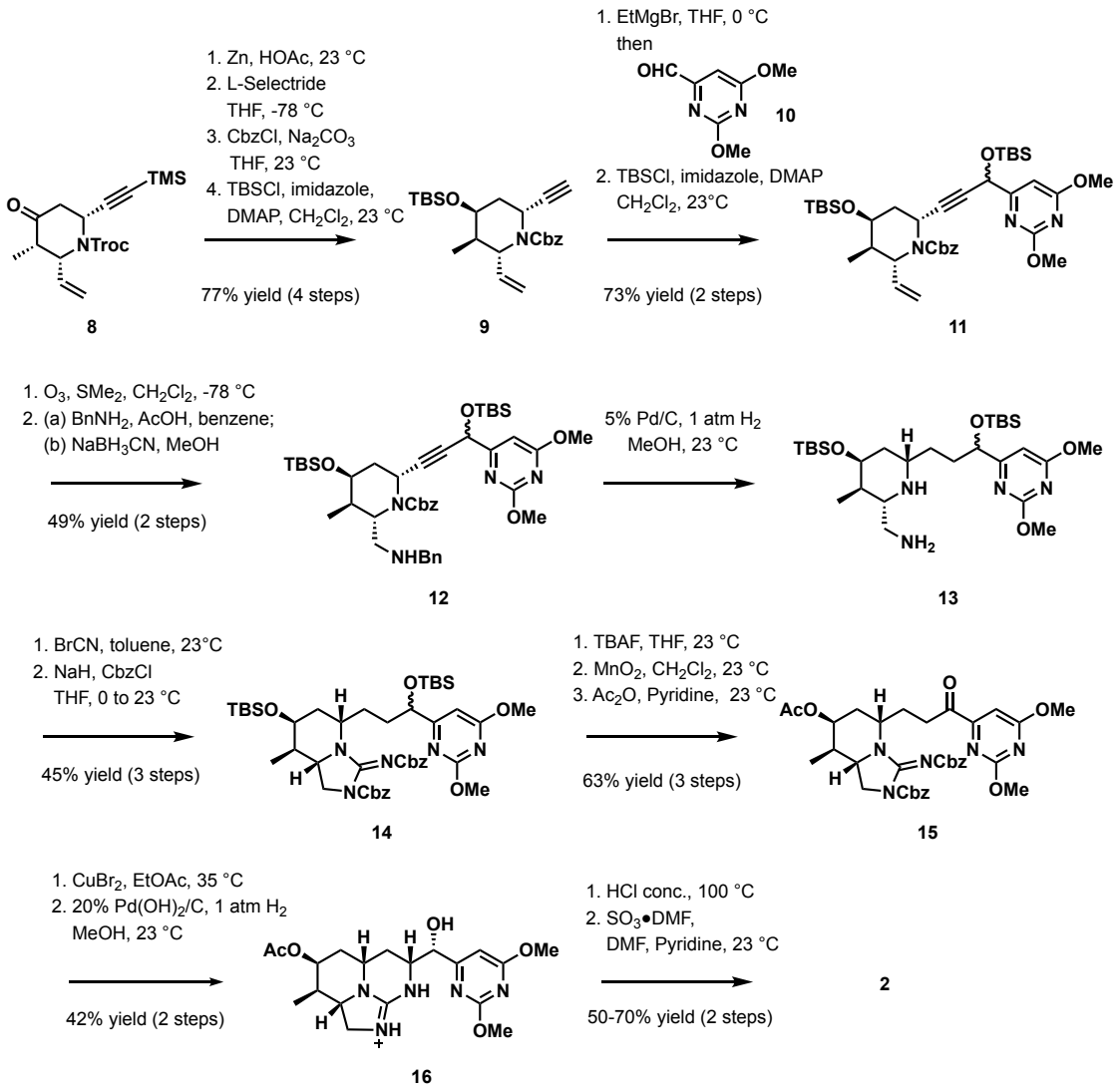
In 2000, Snider *et al* completed the first total synthesis of racemic 7-*epi*-cylindrospermopsin in 20 steps from 4-methoxy-3-methylpyridine **6** in 3.5% overall yield.[26] The 4-methoxy-3-methylpyridine **6** starting material was prepared from 3-picoline **5** using a modified literature procedure in 71% yield (Scheme **1**). Regioselective addition of trimethylsilylethynylmagnesium bromide to **6** at C-6 was favored over addition at C-2 due to steric hindrance of the 3-methyl group. The subsequent transformation features a stereoselective copper-catalyzed addition of vinyl magnesium bromide to **7** to afford **8**. The anticipated $A^{1,3}$ allylic strain between the alkynyl and *N*-acyl group would favor an axial attack by the organocuprate reagent at C-2 to the desired isomer **8** (Scheme **1**).

**Scheme 1.** Preparation of chiral piperidinone intermediate from 3-picoline.




**8** was converted to **9** in four additional steps to prepare the alkyne to couple with aldehyde **10** in the presence of ethylmagnesium bromide, and after silylation with *tert*-butyldimethylsilyl chloride (TBSCl) afforded **11**. Ozonolysis cleaved the terminal alkene to provide the corresponding aldehyde, and condensation of the aldehyde with benzylamine followed by reduction of the resulting imine with sodium cyanoborohydride afforded benzylamine **12**. Hydrogenation of **12** reduced the triple bond and hydrogenolyzed the benzyl and Cbz groups to afford diamine **13**. Formation of the 5-membered cyclic guanidine ring was accomplished after treating **13** with cyanogen bromide (BrCN). Protection of the resulting guanidine with benzyl chloroformate (CbzCl) afforded **14** (Scheme **2**).

**Scheme 2.** Snider's racemic synthesis of 7-*epi*-cylindrospermopsin **2**.




In preparation for completion of the last ring of the tricyclic guanidine core, desilylation of **14** was accomplished in the presence of tetrabutylammonium fluoride. The resulting benzylic alcohol was oxidized with manganese dioxide ($MnO_2$) to form the desired ketone, and the secondary alcohol in the six-membered ring was acetylated with acetic anhydride to give **15**. A key step in Snider's synthesis involves α-bromination of the ketone

**15** with $CuBr_2$ in ethyl acetate to set the stage for the construction of the remaining six-membered ring.[27] Hydrogenation cleaved the Cbz groups to afford the free guanidine, which immediately underwent an intramolecular $S_N2$ reaction to complete the structural core of 7-*epi*-cylindrospermopsin. Hydrogenation with $H_2$, $Pd(OH)_2/C$ in methanol then led to reduction of the ketone to afford the desired alcohol **16**.

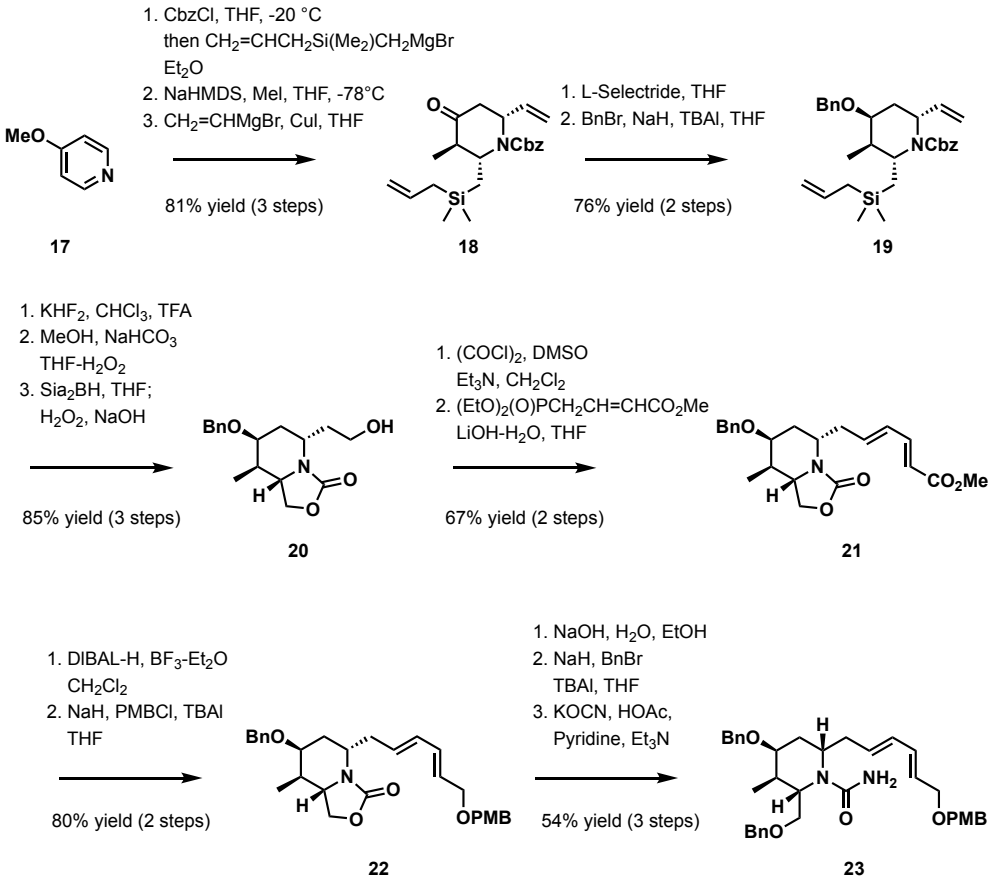
The remaining steps to complete the synthesis of 7-*epi*-cylindrospermopsin involve unveiling the uracil moiety from the dimethoxypyrimidine derivative and monosulfation of the ring alcohol. Hydrolysis of **16** to afford uracil diol was accomplished in concentrated hydrochloric acid. The resulting diol was treated with $SO_3$•DMF in *N*,*N*-dimethylformamide (DMF) and pyridine to give a mixture of 7-*epi*-cylindrospermopsin **2** and bis-sulfated 7-*epi*-cylindrospermopsin.

In 2001, Weinreb and coworkers published their approach for the stereoselective total synthesis of racemic 7-*epi*-cylindrospermposin in 30 steps in 0.1% overall yield.[5] Discrepancy between the spectral data obtained from their synthesis led the group to believe that the original Moore assignment of C-7 stereochemistry for cylindrospermopsin was incorrect, and the structures of **1** and **2** were reversed. Their approach featured a novel stereospecific intramolecular Diels-Alder of an *N*-sulfinylurea heterodienophile[28-30] and application of the group's efficient synthesis of the uracil moiety developed in an earlier report.[31]

The desired Diels-Alder precursor **23** was prepared from 4-methoxypyrimidine **17** (Scheme **3**).[32] *N*-acetylation of 4-methoxypyrimidine **17** was accomplished in the presence of CbzCl. Treatment with (allyldimethylsilyl)methylmagnesium bromide followed by the

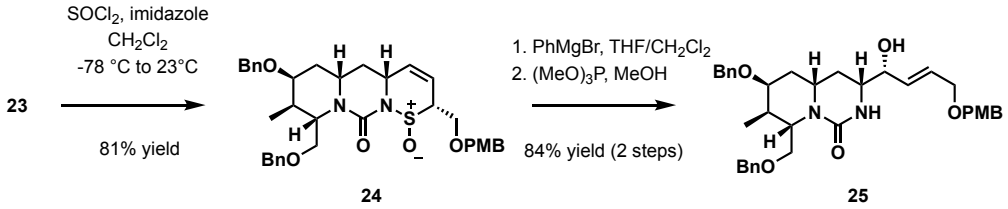
addition of iodomethane in the presence of sodium bis(trimethylsilyl)amide afforded the corresponding enone. Employment of Comins' method established the third desired stereocenter for intermediate **18** via copper-catalyzed conjugate addition of vinylmagnesium bromide.[33] Placement of the vinyl group in the axial position eliminates $A^{1,3}$ allylic strain with the *N*-acyl group. The ketone was reduced with L-selectride, and the resulting alcohol underwent benzylation to afford benzyl ether **19**. Oxidation in the presence of disiamylborane ($Sia_2BH$) followed by an *in-situ* cyclization of the resulting alcohol gave the desired carbamate, and subsequent hydroboration yielded alcohol **20**.

**Scheme 3.** Preparation of Diels-Alder precursor **23**.



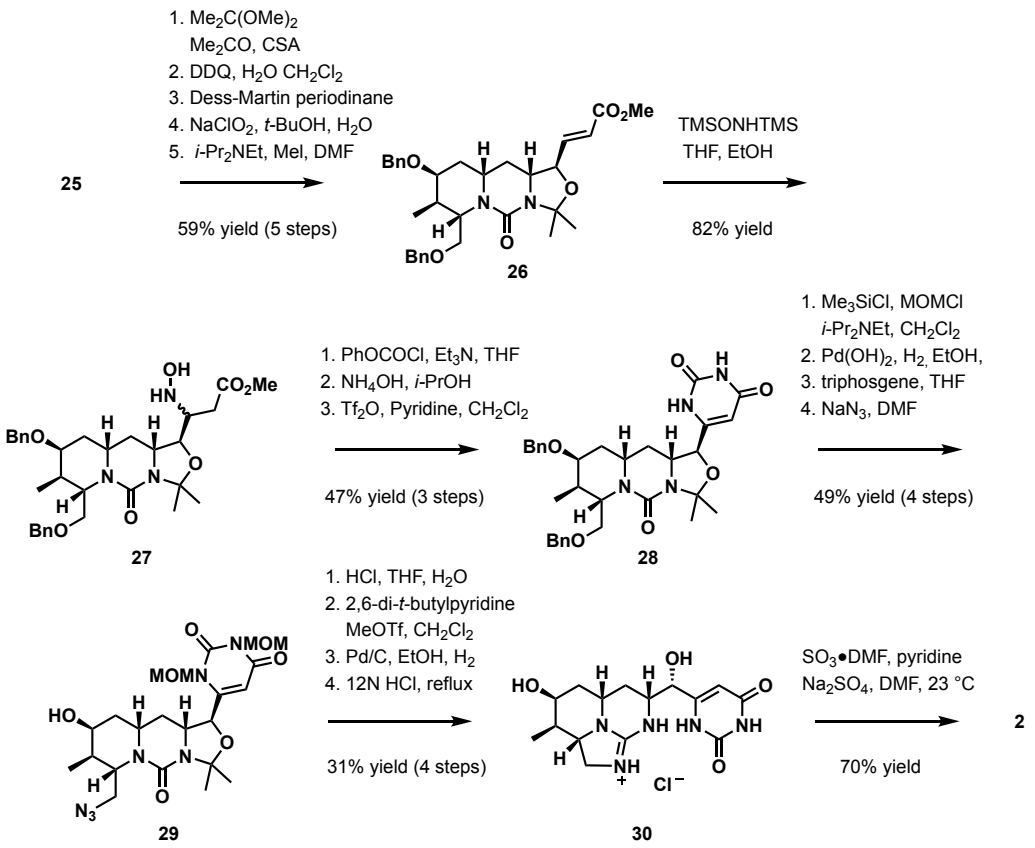
Swern oxidation of alcohol **20** followed by Horner-Wadsworth-Emmon's reaction with (*E*)-methyl-4-(diethoxyphosphoryl)but-2-enoate furnished (*E,E*)-diene ester **21**. The ester group was reduced and protected as the *p*-methoxybenzyl ether **22**. The desired urea Diels-Alder precursor **23** was achieved in 3 additional steps in 54% overall yield from **22** (Scheme **4**).

**Scheme 4.** Formation of (*E*,*E*)-diene ester **25**.




(*E*,*E*)-diene urea **23** was treated with thionyl chloride to afford the tricyclic adduct **24** in excellent yield. Treatment of the adduct with phenylmagnesium bromide followed by trimethyl phosphite afforded a single stereoisomeric allyl alcohol **25**. This intermediate contains all six stereogenic centers present in the 7-*epi*-cylindrospermopsin natural product (Scheme **5**).

**Scheme 5.** Construction of uracil derivative via Weinreb and cowork's established method.

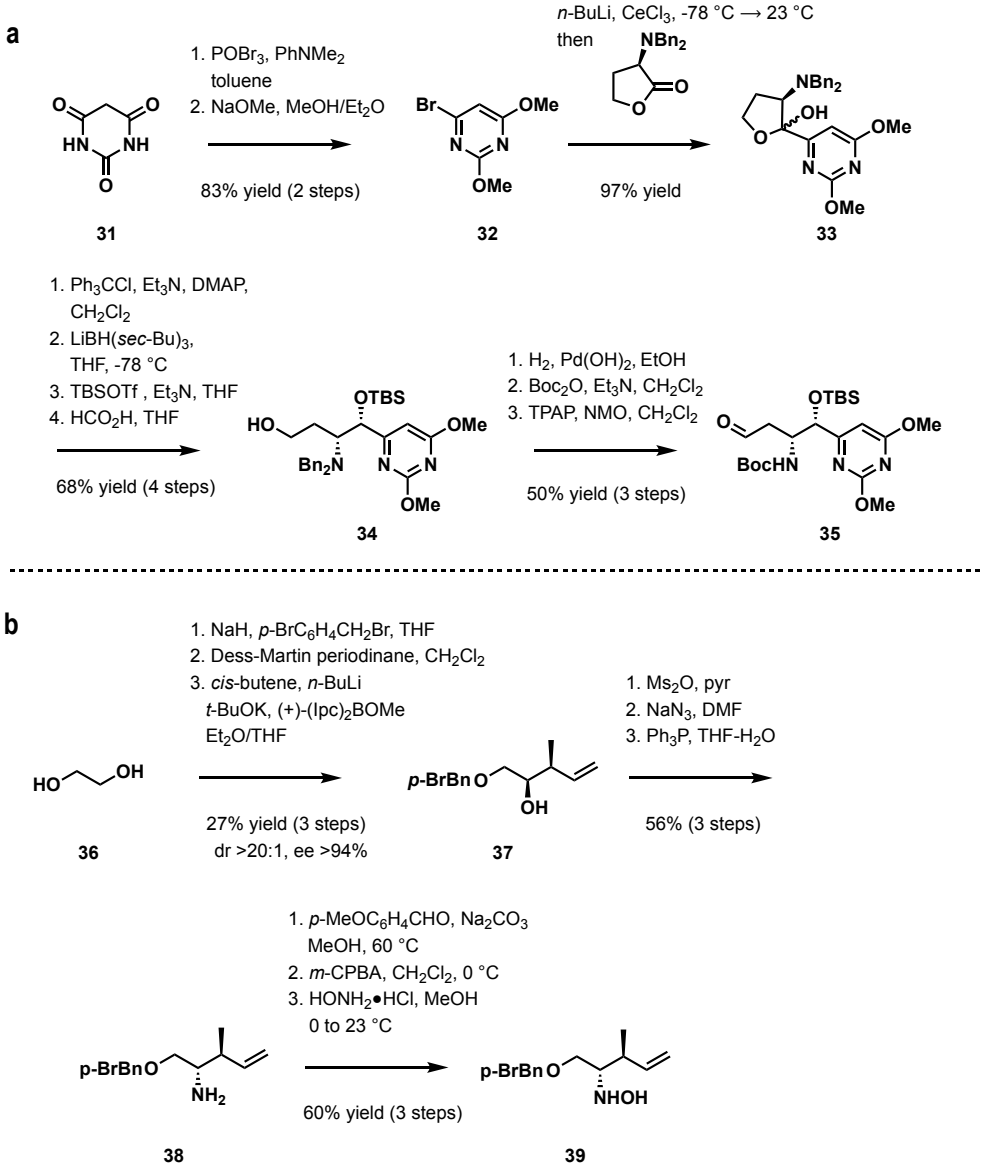



The remainder of the synthesis focuses on the incorporation of the uracil moiety, utilizing the group's strategic approach from α,β-unsaturated esters in a three-step protocol, and the construction of the 5-membered guanidine ring (Scheme **5**). The desired α,β-unsaturated ester **26** was prepared from **25** in 5 steps (59% overall yield). The ester was subjected to conjugation addition of hydroxylamine to ester **27,** which was treated with phenyl chloroformate and then ammonium hydroxide to give the desired *N*-hydroxydihydrouracil. The intermediate was subjected to immediate dehydration in the presence of triflic anhydride to furnish **28** containing the desired uracil ring. To set the stage

for the cyclization to construct the desired guanidine, the tethered *O*-benzyl group was transformed into azide **29**, and reduction of the azide by catalytic hydrogenation led to the direct formation of guanidine **30**. The MOM groups were removed, and monosulfation was achieved with $SO_3$•DMF to complete the synthesis of the natural product **2**.

White and Hansen reported the first asymmetric synthesis of **2** in late 2001.[34] In their synthesis, the group confirmed the absolute configuration of natural 7-epi-cylindrospermopsin. The key step of White and Hansen's synthesis exploited a convergent coupling of aldehyde **35** and hydroxylamine **39** to afford a nitrone intermediate, which would undergo an intramolecular cycloaddition to advance to the desired oxazabicyclo[2.2.1]heptane **40**.[35]

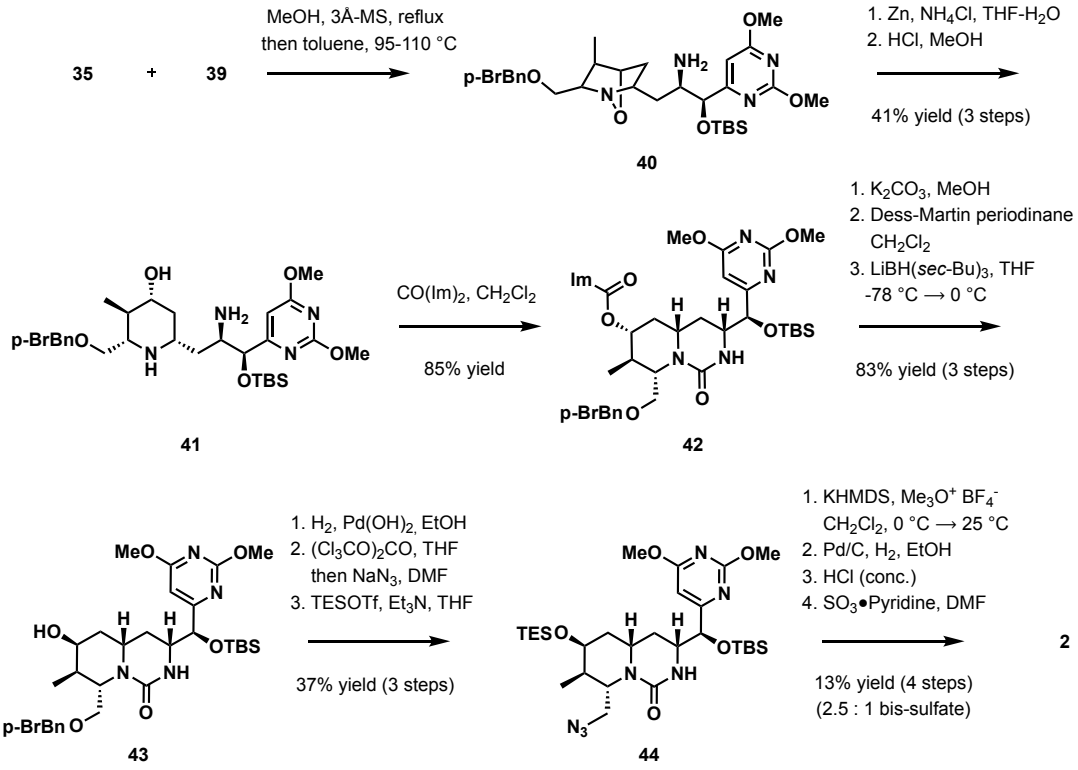
**Scheme 6.** Synthesis of aldehyde and hydroxylamine towards construction of structural core of 7-*epi*-cylindrospermopsin.




Aldehyde **35** and hydroxylamine **39** were prepared independently from barbituric acid **31** (Scheme **6a**) and ethylene glycol **36** (Scheme **6b**), respectively. The two fragments were coupled by refluxing in methanol with 3 Å molecular sieves, then heating the resulting nitrone intermediate to afford oxazabicyclo[2.2.1]heptane **40** (Scheme **7**). N-O cleavage

under reductive conditions followed by carbonylation afforded cyclic urea **42**. Reduction of azide **44** promoted a spontaneous cyclization to afford the tricyclic guanidine core. Global deprotection with concentrated hydrochloric acid and monosulfation in the presence of $SO_3$•DMF afforded **2** in 28 steps in 0.3% overall yield, and the natural product's absolute configuration was confirmed.

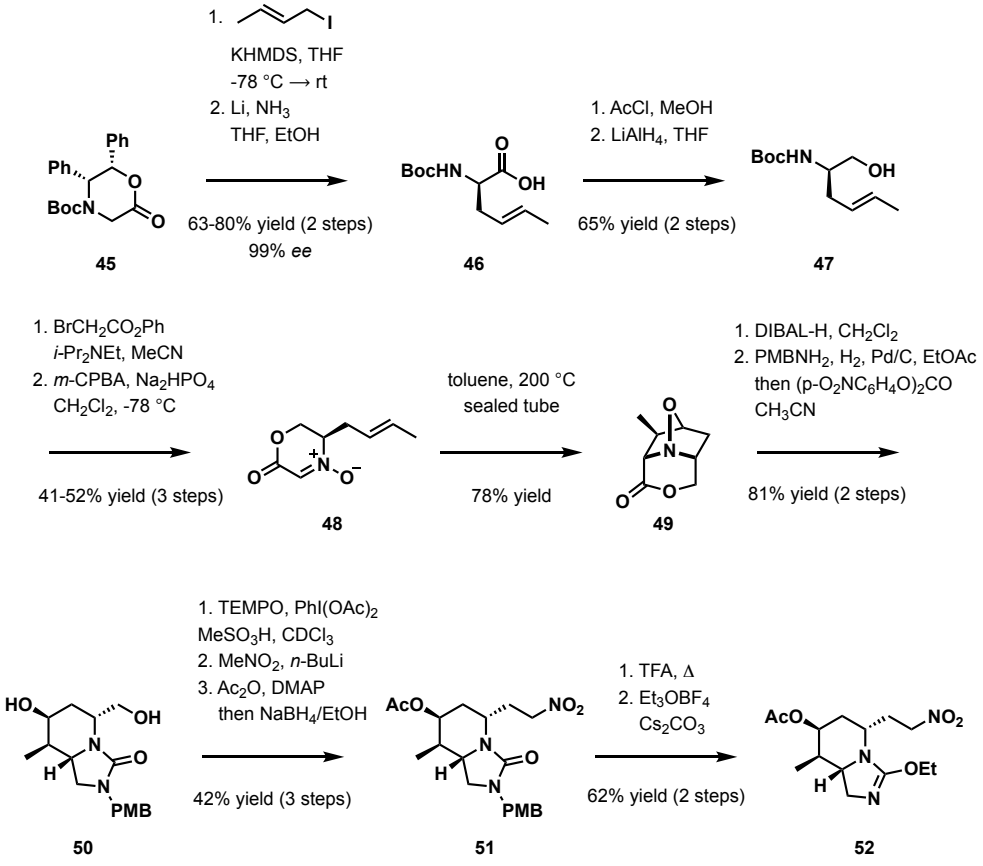
**Scheme 7.** White and Hansen's asymmetric synthesis of 7-*epi*-cylindrospermopsin.




Similar to Hansen and White's approach, Looper and Williams' synthesis of 7-*epi*-cylindrospermopsin utilizes an intramolecular [3+2] cycloaddition of nitrone. In the Looper/Williams synthesis, the [3+2] cyclization was performed at an early stage, and the

convergence point combining **52** and **53** to incorporate the uracil derivative was pursued later in the synthesis. Along with 7-*epi*-cylindrospermopsin, Looper and Williams were also able to access cylindrospermopsin and 7-deoxycylindrospermopsin using their synthetic route.[36-38]

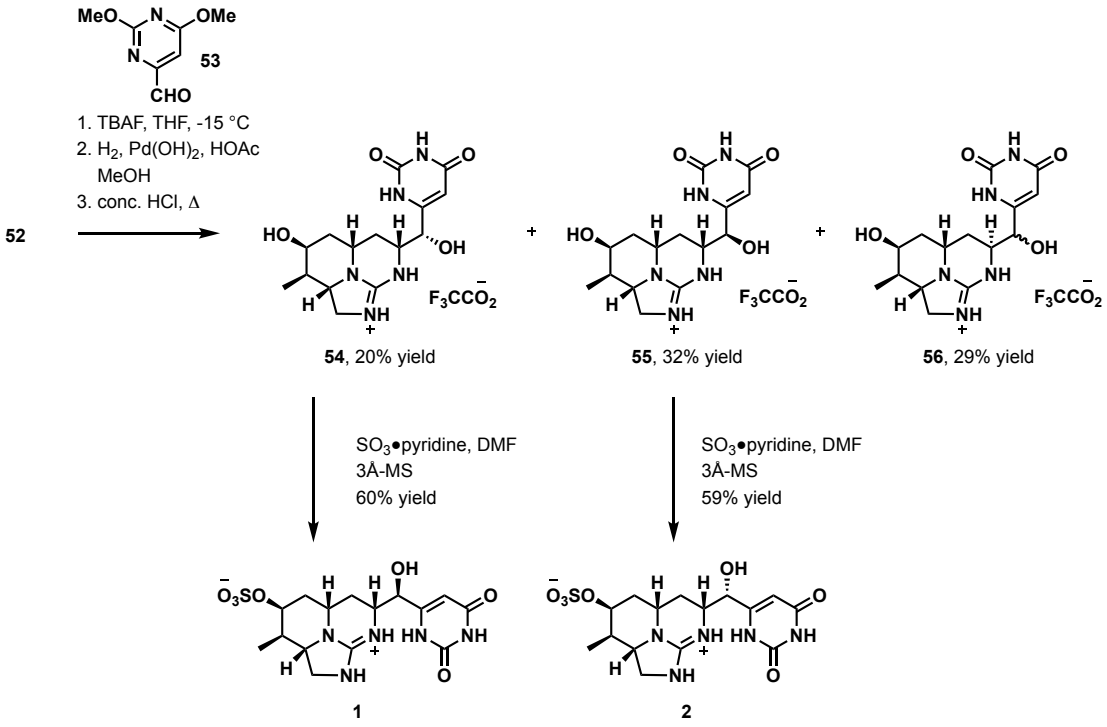
**Scheme 8.** Synthesis of isourea in preparation for coupling with 2,6-dimethoxypyrimidine-4-carbaldehyde to assemble the structural core of 7-*epi*-cylindrospermopsin.




Acid **46** can be prepared from oxazinone **45** in excellent enantiomeric excess via an alkylation with crotyl iodide followed by the removal of the auxiliary with $Li/NH_3$ (Scheme **8**). After reduction of acid **46**, the corresponding primary alcohol **47** was treated with α-

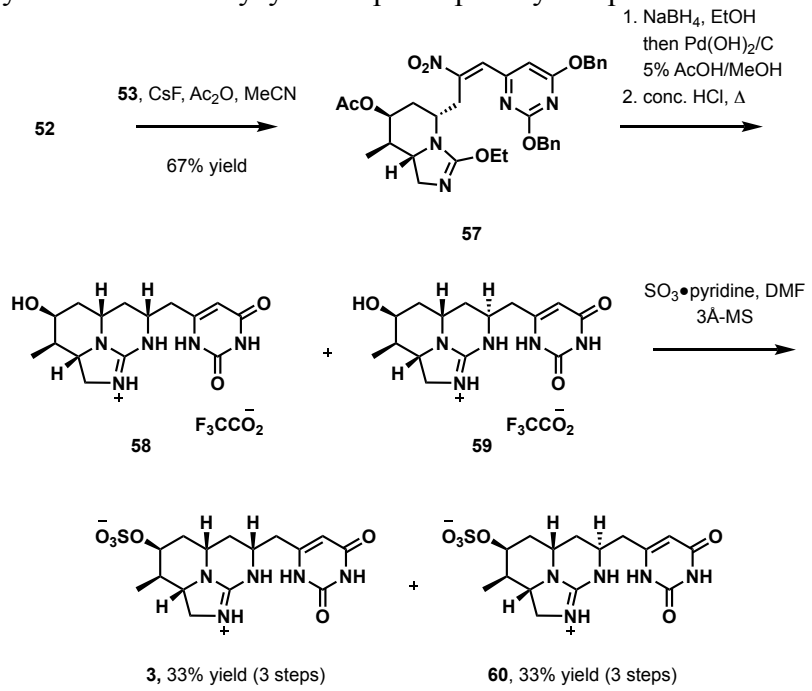
bromophenyl acetate and then with *m*CPBA in dichloromethane to afford oxazinone-*N*-oxide **48**. The desired tricyclic isoxazolidine **49** was generated by heating oxazinone-*N*-oxide **48** in toluene at 200 °C. The carbonyl group in **49** was reduced with diisobutylaluminum hydride (DIBAL-H), and the resulting lactol was reduced to a primary amine under hydrogenation conditions. Treatment of the primary amine with bis(4-nitrophenyl)methanone afforded the desired bicyclic urea **50**. Isourea **52** was prepared from **51** for subsequent coupling with 2,6-dimethoxypyrimidine-4-carbaldehyde **53**.

**Scheme 9.** Synthesis of cylindrospermopsin and 7-epi-cylindrospermopsin by Looper and Williams.



Treatment of **52** and aldehyde **53** with tetrabutylammonium fluoride successfully coupled the two fragments (Scheme **9**). Acidic hydrolysis afforded a separable mixture of epimers **54** and **55**. After separation by HPLC, the epimers were subjected to monosulfation independently to give **1** and **2** in 60% and 59% yield, respectively.

**Scheme 10.** Synthesis of 7-deoxycylindrospermopsin by Looper and Williams.




For the synthesis of 7-deoxycylindrospermopsin, isourea **52** was treated with aldehyde **53** in the presence of acetic anhydride and cesium fluoride to afford nitroalkene **57** (Scheme **10**). The resulting intermediate was subjected to a one-pot conjugated reduction and reductive guanidinylation sequence to give diastereomers **58** and **59**. Sulfation at the cyclic secondary alcohol afforded 7-deoxycylindrospermopsin **3** in 33% yield and a corresponding diastereomer **60** in 33% yield.

Given the structural complexity of the guanidine-uracil alkaloid, a promising approach would be to simply adopt an existing total synthesis method disclosed in the literature. However, adoption for the incorporation of $^{15}N$ isotopes creates difficulties, with each individual synthesis requiring 23-30 steps, along with uncertainties regarding the scalability of each synthetic sequences. These key concerns prompted the development of a *de novo* approach, with the effective introduction of $^{15}N$-isotope as a major objective.

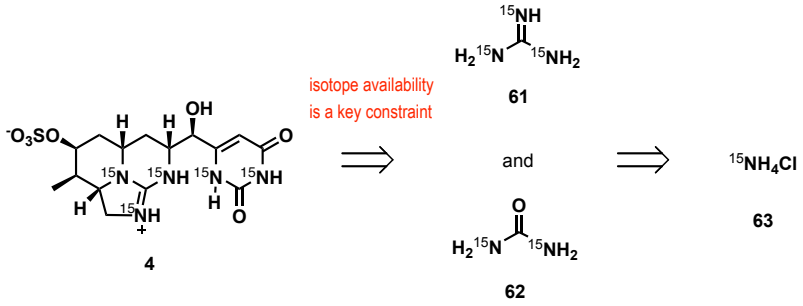
## Chapter 2: Total synthesis of isotopically-labeled cylindrospermopsin

### Total synthesis of [$^{15}N_5$]-cylindrospermopsin

Several principal considerations factored into the synthesis planning. Compared to a typical total synthesis effort, the payoff of the convergent versus linear, atom-by-atom assembly is now substantially enhanced because all but the most basic sources of the $^{15}N$ isotope ($^{15}NH_4Cl$ or aqueous $^{15}NH_3$) become prohibitively expensive, especially in the amounts required for a total synthesis application. A design with preassembled intermediates containing several $^{15}N$ isotopes would be advantageous. Undoubtedly, the common metrics of synthetic efficiency is still applied: minimizing the number of individual synthetic steps based on robust, reproducible chemical transformations is a timeless goal, which only becomes more critical given the costs of the starting isotopes. In addition, all intended transformations must guarantee retention of isotopic label.

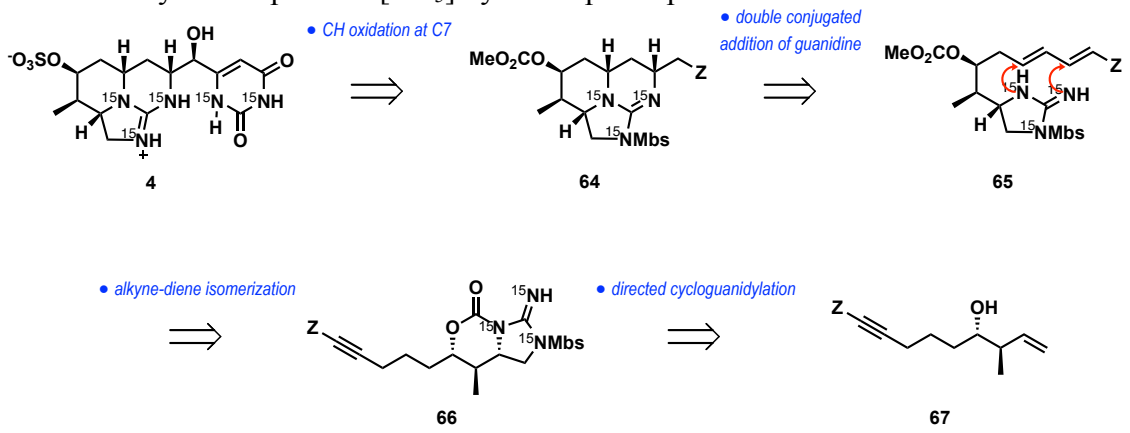
**Retrosynthetic analysis**

**Scheme 11.** Retrosynthetic analysis depicting source of $^{15}N$ isotopes that will be derived from commercially available [$^{15}N$]-ammonium chloride.




The synthesis plan depicted in Scheme **12** was formulated. $^{15}N$ isotopes were envisioned to arise from [$^{15}N_3$]-guanidine **61** and [$^{15}N_2$]-urea **62**, each derived from $^{15}NH_4Cl$ **63** and serving as precursors to the tricyclic guanidine and uracil subunits of cylindrospermopsin, respectively (Scheme **11**). The synthesis plan requires a late-stage conversion of the electron-withdrawing group **Z** in intermediate **64** to uracil and C–H oxidation at C-7. The electron-withdrawing group **Z** enables a key simplification to diene **65** by disconnection of two C–N bonds in the tricyclic guanidine core at C-8 and C-10. Stereoselective intramolecular double conjugate addition of guanidine to diene is expected to ensure rapid assembly of the tricyclic guanidine subunit of cylindrospermopsins. Alkyne **66** was intended as a precursor to diene **65** via a transition metal-mediated rearrangement. As a final element of the plan, homoallylic alcohol **67** serves as a substrate for a hydroxyl-directed stereoselective delivery of free [$^{15}N_3$]-guanidine that we previously developed for this application.[39]

**Scheme 12.** Synthesis plan for [$^{15}N_5$]-cylindrospermopsin.

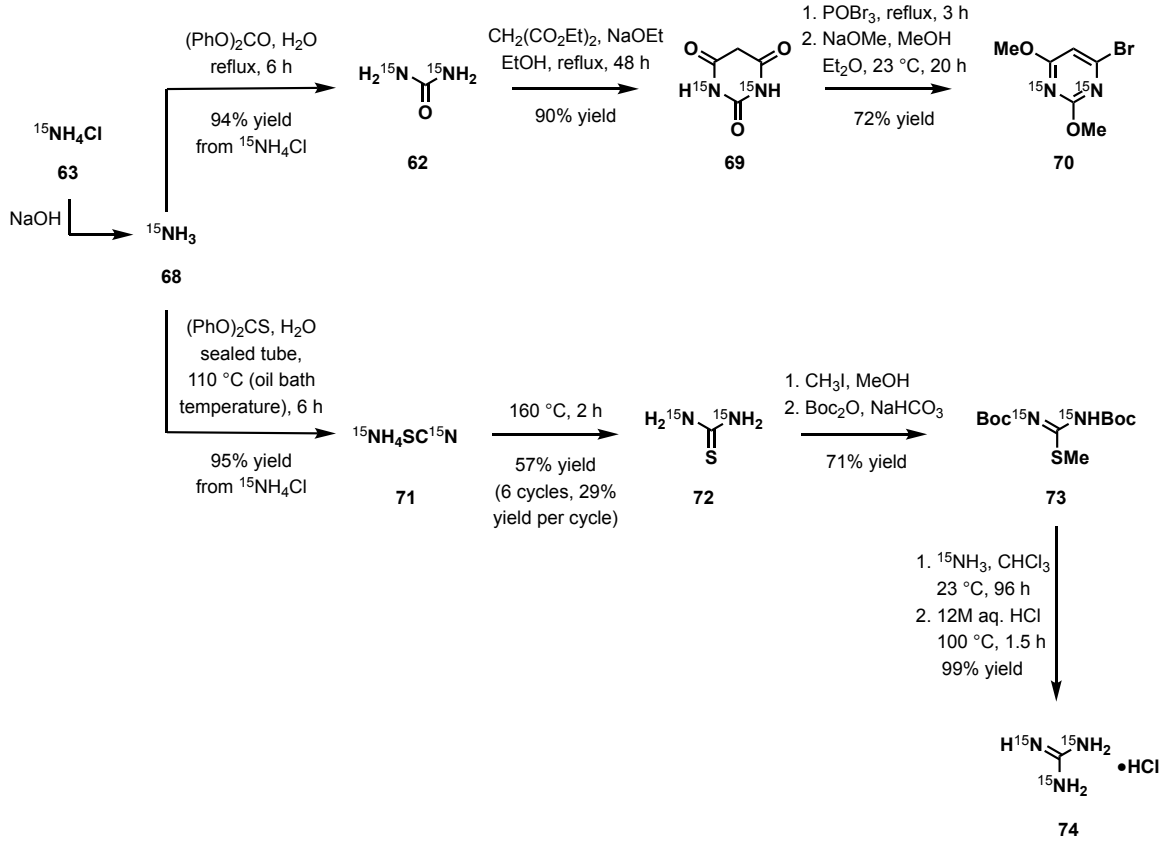



The success of this plan rests on identifying a suitable electron-withdrawing group **Z**, which must first activate the diene for double conjugate addition of guanidine, then facilitate C-7 hydroxylation, and eventually be converted to uracil. Although many options could be considered, heterocyclic substituents like 2,4-dimethoxypyrimid-6-yl appeared to be especially enticing as precursors of uracil. Various electron-deficient heterocyclic groups are known to stabilize carbanions, and activation of double bonds for conjugate addition by substitution with heterocycles has been reported.[40] What remained uncertain was whether *diene* activation for double conjugate addition by guanidine, a centerpiece of our strategy, was feasible. After a careful analysis supported by significant experimentation, we ultimately chose to pursue 2,4-dimethoxypyrimid-6-yl as group **Z** because of its potential to meet these criteria most effectively.

While the initial validation of the entire total synthesis was carried out with the natural isotope, we performed the scaled synthesis starting with [$^{15}$N]-ammonium chloride ($^{15}NH_4Cl$ **63**) in Scheme **13**. [$^{15}$N]-Ammonium chloride proved to be the most economical source of dry liquid [$^{15}$N]-ammonia ($^{15}NH_3$ **68**) on the laboratory scale, affording >95%

yields of the reagent reproducibly upon treatment with sodium hydroxide in solid phase at scales above 0.5 g. [$^{15}N_2$]-2,4-Dimethoxy-6-bromopyrimidine **70** was obtained by the initial conversion of $^{15}NH_3$ to urea **62**, its condensation with diethyl malonate to [$^{15}N_2$]-barbituric acid **69**, treatment with phosphorous oxybromide ($POBr_3$), and methanolysis with two equivalents of sodium methoxide. In contrast to the synthesis of urea, the reaction of $^{15}NH_3$ with diphenyl thiocarbonate gave ammonium thiocyanate, pyrolysis of which was required to access [$^{15}N_2$]-thiourea **72**. The preparation of [$^{15}N_3$]-guanidine hydrochloride **74** was accomplished in four steps by *S*-methylation, treatment with di-*tert*-butyl pyrocarbonate ($Boc_2O$), displacement of methylsulfide group with $^{15}NH_3$ in chloroform, and complete hydrolysis with 12M hydrochloric acid at reflux. The resulting [$^{15}N_3$]-guanidine hydrochloride **74** together with [$^{15}N_2$]-2,4-dimethoxy-6-bromopyrimidine **70** constitute the source of all $^{15}N$ isotopes in the target alkaloids.

**Scheme 13.** The preparation of [$^{15}N_2$]-2,4-dimethoxy-6-bromopyrimidine and [$^{15}N_3$]-guanidine hydrochloride from $^{15}NH_4Cl$, the key precursors constituting the source of all $^{15}N$ isotopes in cylindrospermopsins.

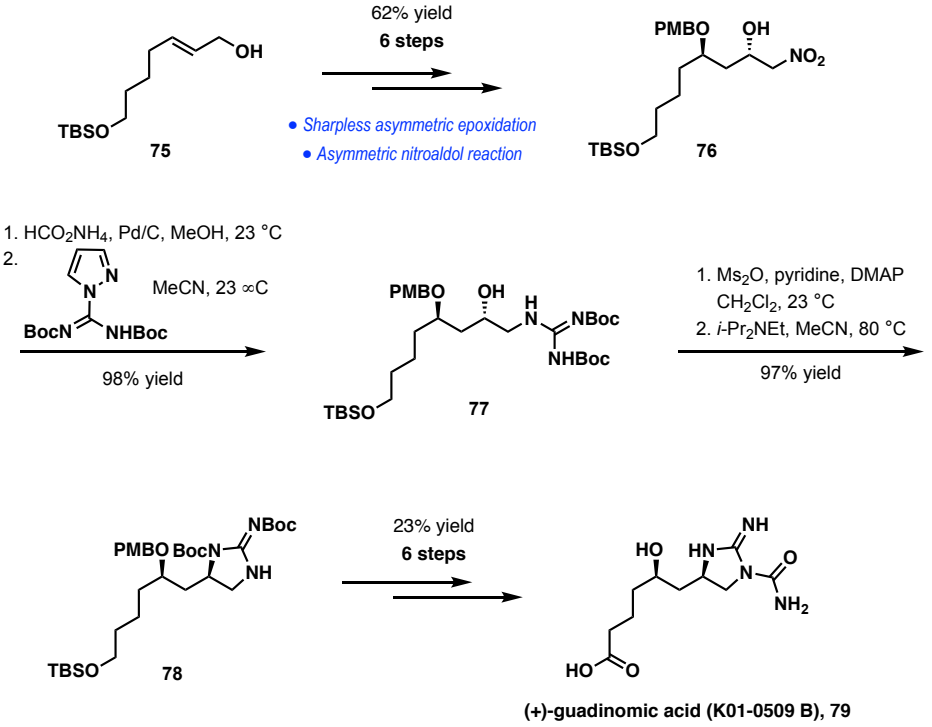



## Construction of 5-membered cyclic guanidine ring

In 2006, Omura *et al* reported their synthetic approach to (+)-guandinomic acid (K01-0509) **75**, a natural product believed to be a plausible biosynthetic intermediate to other guadinomines that have demonstrated to be inhibitors of type III secretion systems. Their approach featured a four-step protocol to assemble the 5-membered cyclic guanidine ring present in the natural product (Scheme **14**).[41] However, previous steps utilizing Sharpless asymmetric epoxidation as well as an asymmetric nitroaldol reaction in the presence of a chiral salen cobalt catalyst is required to set the appropriate stereocenters for the construction

of cyclic guanidine ring to proceed with the desired stereochemical outcome. Furthermore, adopting Omura's approach in the synthesis of cylindrospermopsin proved difficult for the installation of the methyl group present in the natural product.

**Scheme 14.** Omura's approach to incorporate 5-membered cyclic guanidine moiety.

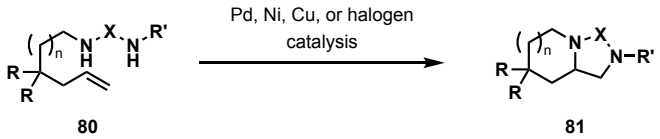



Guanidines are challenging targets in organic synthesis. Present methods for the introduction of the guanidyl group are limited and generally involve guanidinylation of a preinstalled amino group. For these reasons, stereoselective guanidinylation methods are seemingly more difficult and extremely scarce in the literature. Methods in present literature include coupling of primary amines with cyanamide,[42-46] pyrrazolylcarrboxylamidines,[47-49] *S*-alkylisothiourea reagents,[50-60] or trifluromethanesulfonyl guanidine reagents (Goodman reagent).[61-62] Implementation of these strategies in the synthesis of complex guanidine-

containing compounds and natural products can require extended synthetic routes, and in some cases reduction of the overall efficiency of the synthesis.

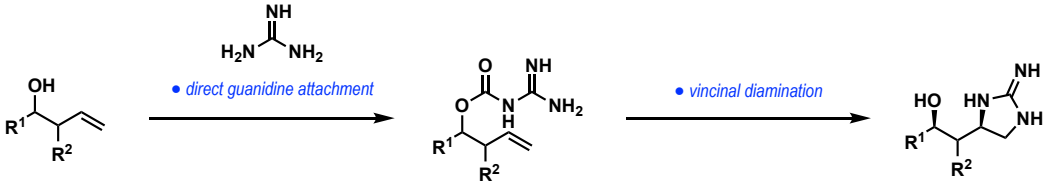
**Scheme 15.** Previous approaches utilizing metal-mediated or halogen catalysis for diamination of unactivated alkenes. Our work entails attachment of guanidine to a hydroxyl group followed by diamination across a tethered alkene to furnish the 5-membered cyclic guanidine moiety.

Previous reports:




80 81

Our work:

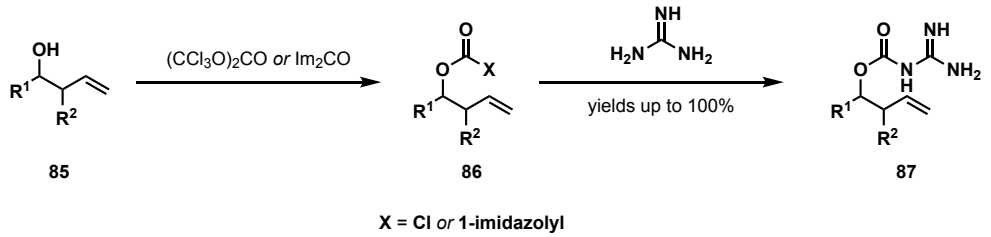



82 83 84

Several methods for inter- and intramolecular vicinal diamination of unactivated alkenes with ureas, sulfamides, and guanidines catalyzed by palladium,[63-74] nickel,[75] copper,[76-83] gold complexes[84-85] and halogenating reagents[86-93] have been reported (Scheme **15**). These promising approaches have yet to demonstrate a broad scope and applicability for stereoselective synthesis of cyclic guanidines with desired functionalization. The requirement for tethering of guanidine or another dinitrogen source by a simple alkyl chain complicates further functionalization of products, and still generally requires guanidilation of a preinstalled amino group at an earlier stage. Realizing that there is a library of natural

products containing the cyclic guanidine moiety prompted us to investigate a new method for efficient stereoselective delivery of an intact guanidine group to an unactivated alkene.

**Scheme 16.** Preparation of acyclic guanidine substrate from corresponding homoallylic alcohol via attachment of carbonyl linker follow by treatment with guanidine.




**85** **86** **87**

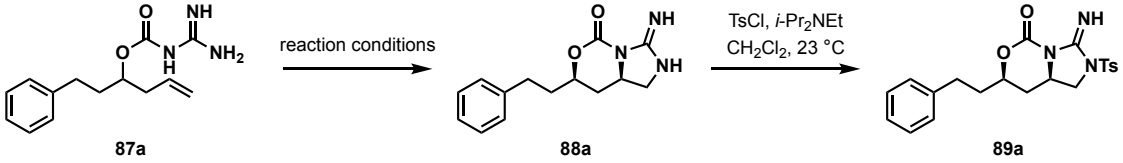
**X = Cl *or* 1-imidazolyl**

As depicted in Scheme **16**, a method for the direct stereoselective guanidinylation of alkenes is described. The guanidine unit can be delivered as an intact fragment by a hydroxy or carboxy group via electrophilic cyclization onto the alkene (Scheme **16**). We found that carbamates **87** can be prepared from alcohols **85** via initial conversion to chloroformates **86** with either triphenylphosgene or with 1,1'-carbonyldiimidazole (CDI) and subsequent treatment with guanidine. In both cases, formation of the acyclic guanidine substrate was comparable using triphosgene and CDI. However, CDI was a more benign and user-friendly reagent due to a high toxicity and volatility of triphosgene. For this purpose, CDI was selected to access our substrate scope for the study of intramolecular delivery of guanidine to a tethered alkene.

The next task was to identify the optimal conditions for intramolecular delivery of the guanidine unit across the tethered alkene. Despite previous success in diamination and cycloguanidylation of alkenyl guanidines mediated by metal catalysis, low conversions and

absence of cyclization product were observed under varying conditions with catalysts such as $Pd(OAc)_2$, $NiCl_2$, or CuI (Table **1**, entries 1-5). In addition to metals, reaction with **88** in the presence of halogen-based reagents has also been attempted. While reaction with tert-butylhypochloride, dipyridineiodonium tetrafluoroborate, and iodine resulted in 100%, 65%, and 15% conversion, respectively, the desired cyclized product was not observed (entries 6-8). Electrophilic cyclization was effected in the presence of *N*-iodosuccinimide (NIS). This reaction performed under optimal conditions, with sodium bicarbonate in acetonitrile at 0 °C, enabled full conversion to the desired product, isolated in 84% yield (entry 10).

**Table 1.** Identification of optimal reagent for cycloguanidylation.




**87a** **88a** **89a**

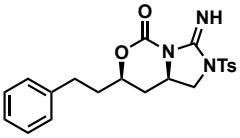
| Entry | reaction conditions | conversion (%) | yield (%) |
|---|---|---|---|
| 1 | 10 mol% $Pd(OAc)_2$, CuI, $K_2CO_3$, DMF, 23 °C, 24 h | 0 | 0 |
| 2 | 25 mol% $Pd(OAc)_2$, $PhI(OAc)_2$, $CH_2Cl_2$, 23 °C, 24 h | 15 | 0 |
| 3 | 5 mol% $Pd(OAc)_2$, $PhI(OAc)_2$, $NMe_4Cl$, NaOAc $CH_2Cl_2$, 23 °C, 24 h | 30 | 0 |
| 4 | 10 mol% $NiCl_2$, $PhI(OAc)_2$, NaOAc, DMF, 23 °C, 24 h | 0 | 0 |
| 5 | 10 mol% CuI, $K_2CO_3$, 10 mol%, 2,2'-bipyridine, $O_2$ DMF, 60 °C, 24 h | 0 | 0 |
| 6 | 3 equiv. *t*-BuOCl, $CH_2Cl_2$, 0 °C, 20 min | 100 | 0 |
| 7 | $Py_2IBF_4$, toluene, 23 °C or reflux, 24 h | 65 | 0 |
| 8 | 2.1 equiv. $I_2$, $NaHCO_3$, $CH_2Cl_2$, 23 °C, 6 h | 15 | 0 |
| 9 | 2.1 equiv. NIS, $NaHCO_3$, $CH_2Cl_2$, 23 °C, 1 h | 100 | 62 |
| **10** | **2.1 equiv. NIS, $NaHCO_3$, $CH_3CN$, 0 °C, 5 h** | **100** | **84** |

Next, the reaction scope was investigated; the substrate scope was designed to test functional group compatibility suitable for applications in medicinal chemistry and natural product synthesis (Table **2**). The cyclized guanidine products were isolated after guanidine sulfonylation to ease purification. *N*-nosylation, *N*-tosylation and *N*-(4-methoxyphenyl)sulfonylation afforded excellent as well as comparable isolated yields **89a-89c**. Tosylation was applied for the substrate scope studies. Compounds with various linear and branched alkyl substituents undergo cyclization, producing expected guanidines **89d-89f** in excellent yields as single diastereomers. The introduction of a benzyl or silyl ether or an ester group as a side chain in substrates did not hamper the overall efficiency of guanidinylation, affording **89g-89j** after tosylation.

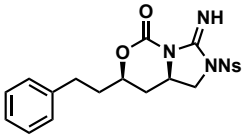
To further investigate the chemoselectivity of the cycloguanidylation method, substrates comprising a distant terminal alkyne or olefin moiety as well as various aryl substituents were examined. Diminished yields were observed in the presence of an alkyne or a second terminal alkene; however, after further investigation, performing the reaction at reduced time (2 h) allowed the isolation of **89k** and **89l** in substantially improved yields, 70% and 50% respectively. Substrates derived from aryl alcohols were observed to be slightly less reactive than their aliphatic counterparts, demonstrated by the formation of **89m-89r**. More notably, cyclization of the guanidine bearing a 4-methoxyphenyl group produced compound **89r** in only 12% yield, accompanied by significant amounts of decomposition products, presumably arising by a competing carbocationic pathway.

**Figure 3.** Substrate scope for the cycloguanidylation reaction.

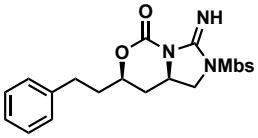
**a**



**89a**
84% yield

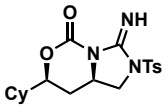


**89b**
85% yield

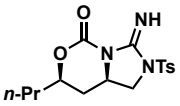


**89c**
79% yield

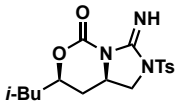
**b**



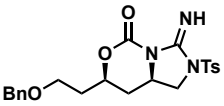
**89d**
79% yield



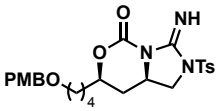
**89e**
86% yield



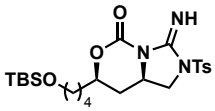
**89f**
91% yield



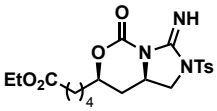
**89g**
77% yield



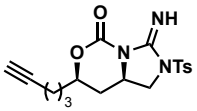
**89h**
87% yield



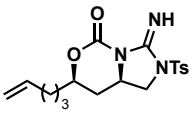
**89i**
84% yield



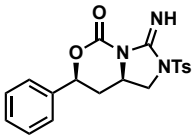
**89j**
71% yield



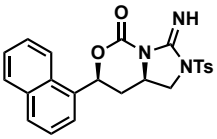
**89k**[a]
70% yield



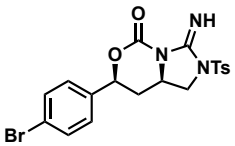
**89l**[a]
50% yield



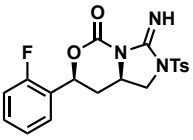
**89m**[b]
72% yield



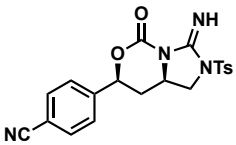
**89n**[b]
50% yield



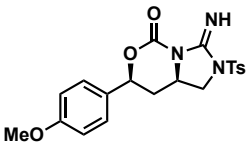
**89o**[b]
87% yield



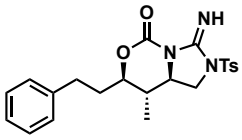
**89p**[b]
72% yield



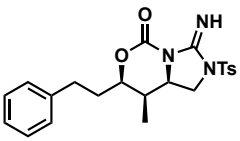
**89q**[b]
43% yield



**89r**[b]
12% yield



**89s**
64% yield
dr 2:1

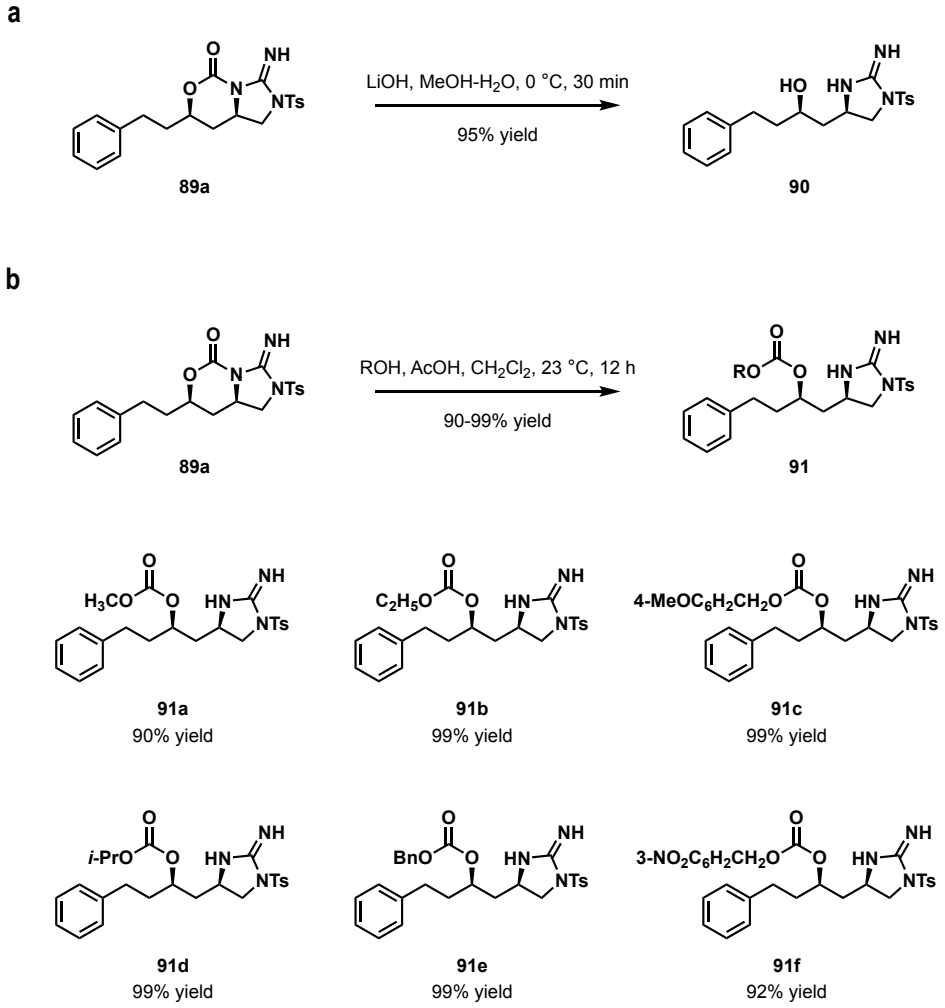


**89t**
70% yield

[a] The reaction was performed at 0 °C for 2 h. [b] The reaction was performed at 0 °C for 5 h then 23 °C for another 5 h.

Furthermore, cyclization of substrates derived from both *syn*- and *anti*-3-methyl-1-alkene-4-ols were also investigated. Reaction of the *syn*-isomer gave rise to **89t** in 70% yield as a single diastereomer (Figure **3**). However, reaction with *anti*-3-methyl-1-alkene-4-ol with NIS produced the cyclic product **89s** in 64% as a 2:1 mixture of diastereomers.

**Figure 4.** Functionalization of cyclic carbamate to afford corresponding alcohol and acyclic carbonates.



Two strategies for the modification of the carbonyl tether were investigated. Rapid hydrolysis of **89a** in the presence of aqueous lithium hydroxide readily furnished the corresponding alcohol **90** in 95% yield (Figure **4a**). Furthermore, our group discovered unprecedented and unexpected reactivity whereby cyclic guanidine-derived carbamates undergo regioselective ring opening of the carbamate to furnish corresponding carbonates **91** under mild reaction conditions in acetic acid with different alcohols (Figure **4b**). A range of simple aliphatic and aromatic alcohols were utilized for this transformation, providing orthogonally protected compounds **91a**-**91f** in excellent yields. However, *tert*-butylalcohol was found to be the only unreactive alcohol, presumably due to steric hindrance.

While the precise mechanistic underpinnings related to the first C-N bond forming event are presently unclear, we hypothesize that the stereochemical outcome is governed by transannular interactions in the developing six-membered ring, maximizing the number of equatorial substituents.

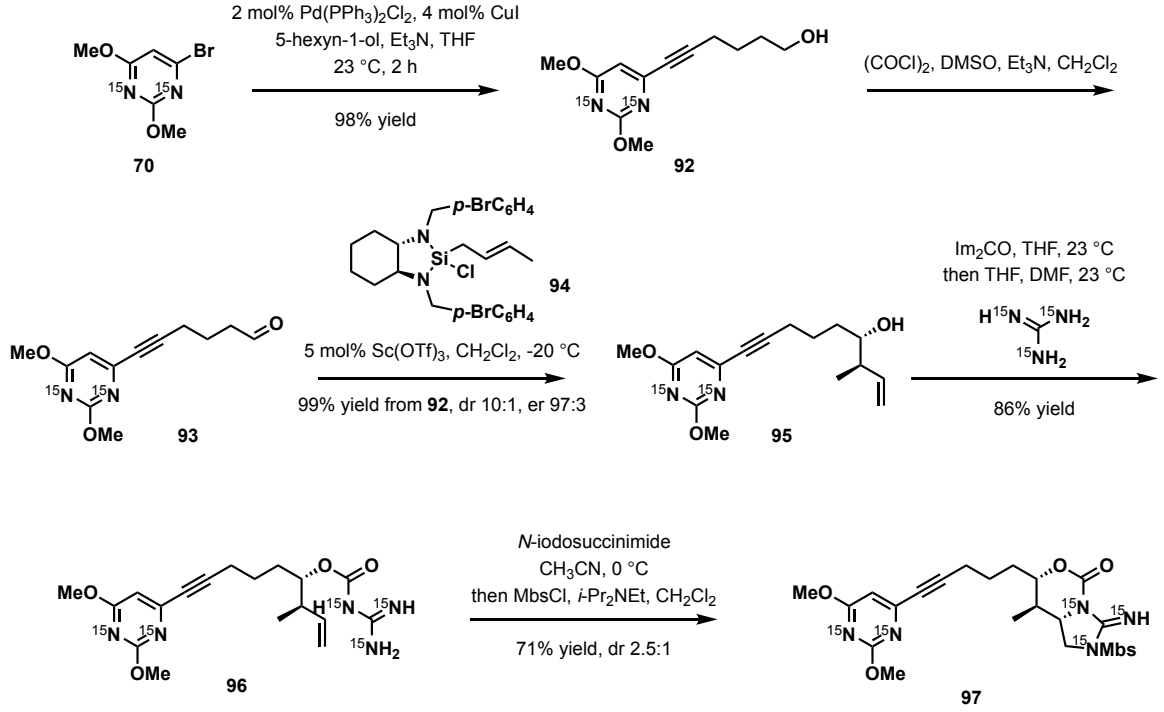
In closing, our group developed a method for directed stereoselective guanidinylation of alkenes. Electrophilic cyclization onto an alkene is accomplished via delivery of guanidine by a carbamate linker with a high level of stereocontrol. After the guanidine delivery, the directing group can be cleaved under exceptionally mild conditions, typically by alcoholysis in the presence of acetic acid. Broad functional group tolerance and mild reaction conditions for the cycloguanidylation and subsequent tether removal or modification suggest applications in medicinal chemistry and natural product synthesis.

**Forward synthesis of cylindrospermopsin alkaloids**

In the forward synthesis of [$^{15}N_5$]-cylindrospermopsin **4**, 6-bromo-2,4-dimethoxypyrimidine **70** undergoes Sonogashira couping with 5-hexyn-1-ol. Alcohol **92** is subjected to Swern oxidation and the resulting aldehyde **93** undergoes enantioselective crotylation with Leighton's reagent **94** (99% yield, dr 10:1, er 97:3). Krische's method has also been pursued to promote direct conversion of **92** to **95** in hopes of shortening our synthesis. Although the enantiocontrol was excellent (er 98:2), lower diastereoselectivity and yield thwarted its application for the scaled synthesis of **95** (Scheme **17**).

The 5-membered cyclic guanidine was assembled using the cycloguanidylation method mentioned previously. Acyclic guanidine **96** was prepared from alcohol **95** with CDI followed by treatment with free guanidine. The cyclic guanidine was furnished via electrophilic cyclization in the presence of *N*-iodosuccinimide, and the product **97** was isolated after treatment with 4-methoxybenzenesulfonyl chloride (MbsCl) in 71% yield and 2.5:1 dr favoring the desired diastereomer.

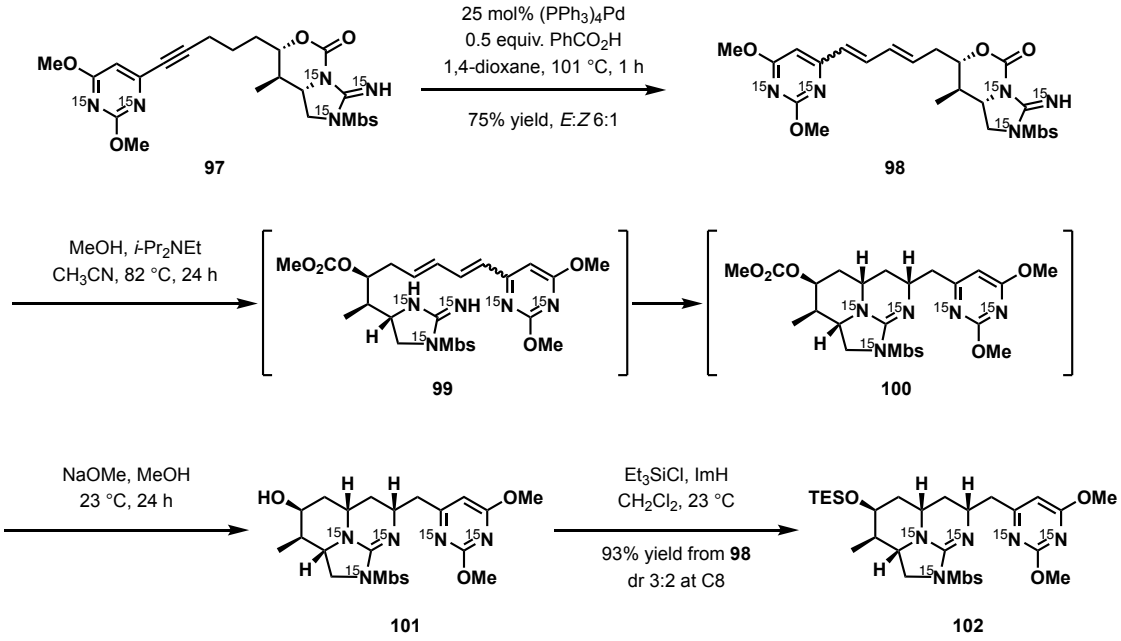
**Scheme 17.** Construction of 5-membered cyclic guanidine moiety using our cycloguanidylation method mentioned previously in the forward synthesis of [$^{15}N_5$]-cylindrospermopsin.




Next, diene **98** was pursued to set the stage for key double conjugated addition that would furnish the tricyclic guanidine core present in the cylindrospermopsin alkaloids (Scheme **18**). Diene **98** is prepared in one step via alkyne to diene isomerization in the presence of catalytic amounts of palladium-tetrakis(triphenylphosphine) and benzoic acid in 75% yield as a 6:1 mixture of *E*- and *Z*- isomers. Initial methanolysis was accomplished by heating **98** with 14% methanol in acetonitrile, leading to opening of the carbamate ring to the corresponding carbonate. Refluxing in the presence of *N*-ethyldiisopropylamine also resulted in the formation of the tricyclic core. In the presence of sodium methoxide, methyl carbonate is removed, and subsequent silylation with chlorotriethylsilane (TESCl) afforded

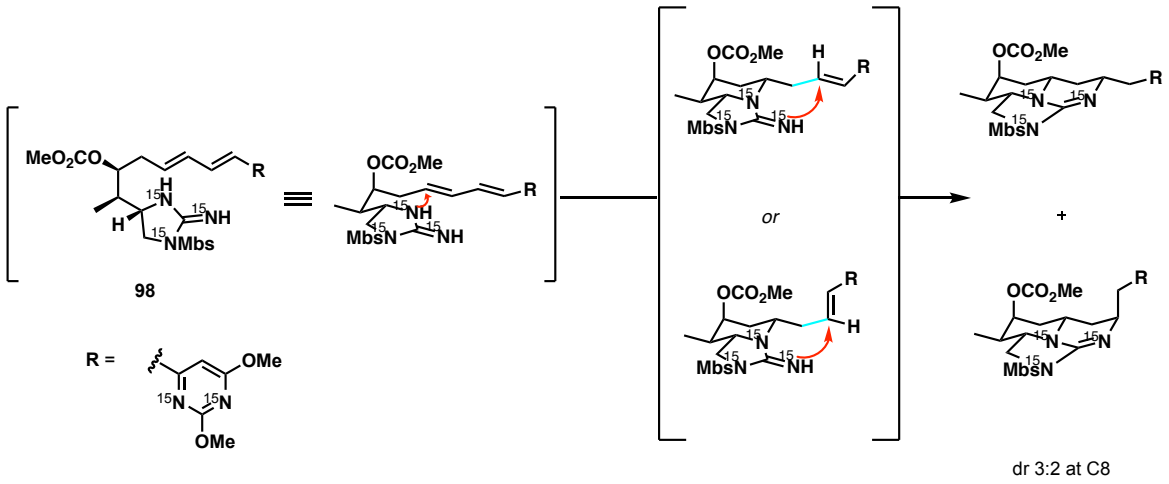
**102** in 93% overall yield from diene **98** as a separable 3:2 mixture of diastereomers at C-8 favoring the requisite *R* configuration.

**Scheme 18.** Assembly of the structural core of cylindrospermopsin via double conjugated addition.



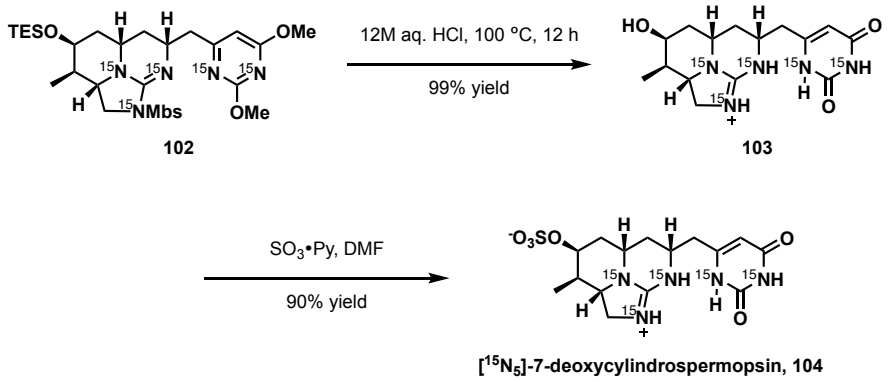

We propose that formation of the first six-membered ring undergoes a chair transition state, which maximizes the number of substituents in the equatorial position while minimizing steric interaction between the methyl group and the methyl carbonate group, therefore resulting in the formation of single isomer. However, formation of the second six-membered ring in the tricyclic system, with the ability to rotate about the highlighted sigma bond, allows for the formation of two diastereomers (Scheme **19**).

**Scheme 19.** Formation of diastereomers at C-8.



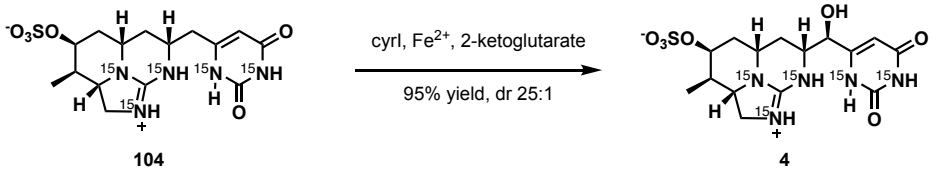

The advantage of substitution of methyl carbonate with TES was not apparent initially. However, in doing so we were able to advance in our synthesis with intermediate **102** which enabled us to perform simple chromatographic separation of C-8 diastereomers. The substrate withstood basic conditions required for the ensuing C-7 metallation and oxidation. Our synthesis thus far has delivered 1.55 g of **102**, a portion of which was subjected to reflux in 12 M hydrochloric acid, resulting in clean hydrolysis of Mbs sulfonamide, triethylsilyl ether, and dimethoxypyrimidine affording guanidine uracil **103** in 99% yield. A straightforward *O*-sulfonation was accomplished with $SO_3$•Py complex in DMF (90% yield) completing the synthesis of [$^{15}N_5$]-7-deoxycylindrospermopsin **104** (Scheme **20**).

**Scheme 20.** Synthesis of [$^{15}N_5$]-7-deoxycylindrospermopsin.




Two approaches based on biological or chemical oxidation were pursued for incorporation of the hydroxyl group at C-7 toward [$^{15}N_5$]-cylindrospermopsin **4**. Biooxidation represents the last step in the biosynthesis of cylindrospermopsin catalyzed by CyrI, a 2-oxoglutarate dependent non-heme iron oxygenase (Scheme **21**). We succeeded in expressing significant quantities of CyrI (Oscillatoria strain PCC7926) in *E. coli* and applying the enzyme for the direct biooxidation of [$^{15}N_5$]-7-deoxycylindrospermopsin under aqueous conditions, adapting the protocol reported by Ploux for 8.2 mg scale.[94] The enzyme showed high selectivity for cylindrospermopsin, affording the product in 95% yield and better than 25:1 diastereoselectivity. At higher loadings of the enzyme, selectivity tended to improve to >90:1. However, we found the practical utility of this oxidation limited by very low solubility of [$^{15}N_5$]-7-deoxycylindrospermopsin, found to be approximately 44 mg/L. Notably, no oxidation was observed with desulfated substrate **103**.

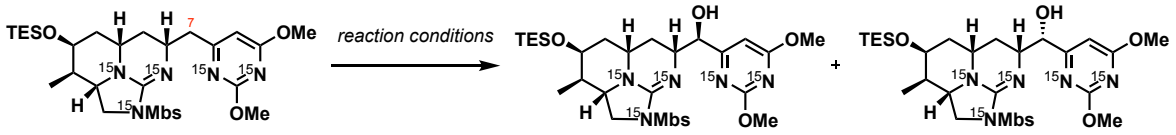
**Scheme 21.** Biooxidation of [$^{15}N_5$]-7-deoxycylindrospermopsin to access [$^{15}N_5$]-cylindrospermopsin.




104 4

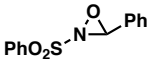
Chemical oxidation at C-7 was also pursued with **102** to improve scalability (Table **2**). The plan was to utilize the anion-stabilizing capacity of pyrimidine for C-7 metallation followed by subsequent hydroxylation of the carbanion. Rapid lithiation of **102** with lithium diisopropylamide (LDA) followed by quenching with an oxaziridine afforded the C-7 hydroxylation products in good yields along with recovered starting material. The diastereomeric ratio is dependent on the oxaziridine: **107** afforded a 6:1 dr, while camphor based reagents **108** and **109** gave >20:1 dr in favor of **105**. The optimized hydroxylation was scaled to 1.50 g of **102**, providing **105** as a pure isomer in **46**% yield (and **41**% recovered starting material).

**Table 2.** Optimization of reaction conditions for chemical oxidation at C-7.




**102** **105** **106**

| Entry | *reaction conditions* | yield (%) | **105**/**106** (ratio) |
|---|---|---|---|
| 1 | 2 equiv. LDA, THF, -78 °C, 5 min<br>then 3 equiv. **107**, -78 °C, 2 min | 22 | 6:1 |
| 2 | 2 equiv. LDA, THF, -78 °C, 5 min<br>then 3 equiv. **108**, -78 °C, 2 min | 23 | 20:1 |
| 3 | 2 equiv. LDA, -78 °C, 5 min<br>then 3 equiv. **109**, -78 °C, 2 min | 21 | 25:1 |
| **4** | **1.2 equiv. LDA, -78 °C, 5 min<br>then 3 equiv. 109, -78 °C, 2 min** | **51 (84 brsm)** | **25:1** |



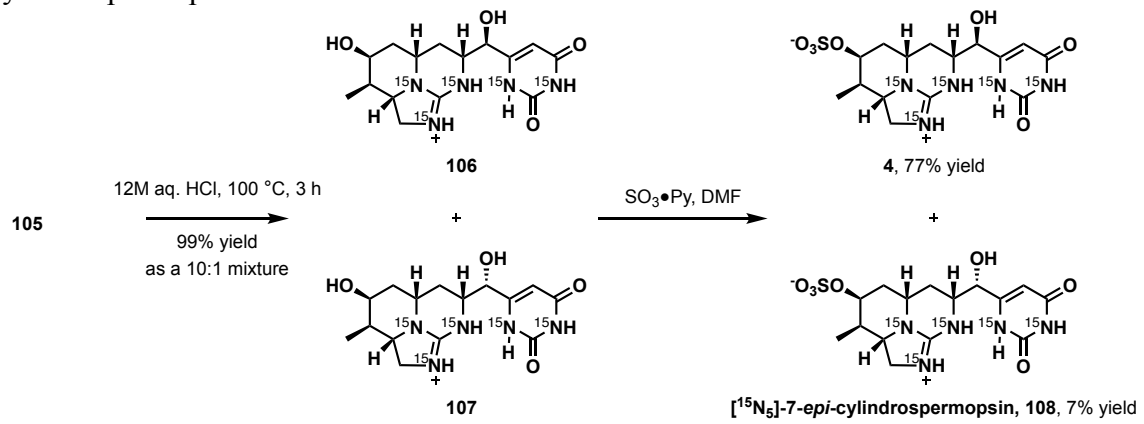

**107**


**108**


**109**

Complete hydrolysis was again achieved by reflux in 12 M hydrochloric acid, although minor epimerization at C-7 was observed during hydrolysis (Scheme **22**). The mixture of epimers **106** and **107** was submitted to *O*-sulfonation with $SO_3$•Py complex in DMF according to known protocol. Selective monosulfation of the diols at the cyclic OH group was observed. The mixture of products was readily separated by reverse-phase HPLC, affording [$^{15}N_5$]-cylindrospermopsin **4** in 77% yield (75 mg) and [$^{15}N_5$]-7-*epi*-cylindrospermopsin **108** in 7% yield (6.8 mg).

**Scheme 22.** Total synthesis of [$^{15}N_5$]-cylindrospermopsin and [$^{15}N_5$]-7-*epi*-cylindrospermopsin.

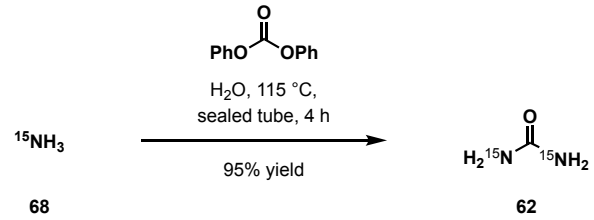



## Conclusion

We have completed the enantioselective total synthesis of isotopically-labeled cylindrospermopsin, 7-*epi*-cylindrospermopsin and 7-deoxycylindrospermopsin. Our synthetic approach both highlighted the effectiveness of our group's stereoselective guanidinylation method in the construction of the 5-membered cyclic guanidine moiety and demonstrated a stereoselective intramolecular double conjugated addition of guanidine to diene leading to the rapid assembly of the tricyclic guanidine core of cylindrospermopsins. In conclusion, ample supply of isotopically-labeled cylindrospermopsin compounds by total synthesis enabled its development as an analytical standard for the precise quantification in raw environmental samples.

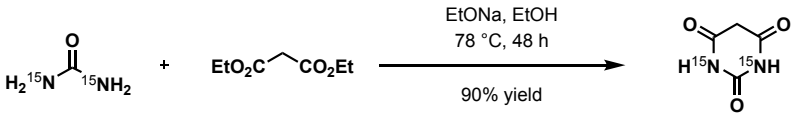
## Experimental Procedures

**General Information.** All reactions were carried out under an inert atmosphere of dry argon in oven or flame-dried glassware, unless the reaction procedure states otherwise. Tetrahydrofuran (THF) and diethyl ether (ether) were distilled from sodium-benzophenone in a continuous still under an atmosphere of argon. Dichloromethane, *N*,*N*-diisopropylethylamine, and acetonitrile were distilled from calcium hydride in a continuous still under an atmosphere of argon. Reaction temperatures were controlled by IKA ETS-D4 fuzzy thermocouples. Analytical thin-layer chromatography (TLC) was performed using pre-coated TLC plates with Silica Gel 60 $F_{254}$ (EMD no. 5715-7) and visualized using combinations of UV, anisaldehyde, ceric ammonium molybdate (CAM), potassium permanganate, and iodine staining. Flash column chromatography was performed using 40-63 μm silica gel (Merck, Geduran, no. 11567-1) as the stationary phase. Reversed-phase chromatography was performed using SiliCycle SiliaSphere PC, C18 monomeric, 25 μm 90 Å functionalized spherical silica gel. Proton nuclear magnetic resonance spectra were recorded at 400, 500, and 600 MHz on Varian Unity Inova spectrometers. Carbon nuclear magnetic resonance spectra were recorded at 100 MHz, 125 MHz, and 150 MHz on Varian Unity Inova spectrometers. Nitrogen nuclear magnetic resonance spectra were recorded at 41 MHz on Varian Unity Inova spectrometer. All Chemical shifts were reported in δ units relative to tetramethylsilane. High Resolution mass spectral data were obtained using Waters Xevo G2-XS ToF mass spectrometer at the University of California, Santa Barbara. Ammonium chloride ($^{15}N$, 99.7% isotope incorporation) was purchased from Cambridge Isotope Laboratories.



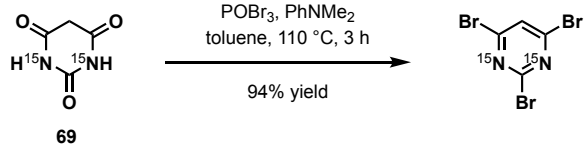

68 62

**Urea-$^{15}N_2$, 62**. This compound was prepared according to the modified method reported by Leitch and Davidson. [95] Ammonium chloride (3.0 g, 55.0 mmol, >99% $^{15}$N isotope incorporation) was mixed with 6 g (0.15 mol) of granular NaOH in a 25 mL one neck round bottom flask equipped with a magnetic stirring bar and an outlet for ammonia connected to a 25-mL round bottom glass pressure vessel cooled to -78°C through the tube, filled with granular sodium hydroxide. The mixture was heated with blow torch until no more ammonia was condensing in the receiving vessel. After the weight of collected ammonia was measured (0.98 g, 54.4 mmol, 99% recovery) it was carefully mixed with water (4.5 mL) at -78 °C. To the frozen mixture diphenyl carbonate (6.54 g, 30.6 mmol) was added and the vessel was sealed. After the mixture was warmed up to room temperature it was placed into an oil bath heated at 115 °C and stirred at this temperature for 4 h. The reaction was cooled to room temperature, diluted with water (50.0 mL) and phenol was removed by extraction with $Et_2O$ (3×40.0 mL). The aqueous phase was separated and concentrated to dryness under reduced pressure. The residual moisture was removed by azeotropic evaporation with methanol to produce title compound (1.605 g, 25.9 mmol, 94% yield based on $^{15}NH_4Cl$) as a white powder. $^1$H NMR (400 MHz, DMSO-$D_6$) δ 5.49 (d, $J$ = 88.6 Hz, 4H, $2NH_2$). $^{13}$C NMR (101 MHz, DMSO-$D_6$) δ 159.87 (t, $J$ = 18.5 Hz). $^{15}$N NMR (41 MHz, DMSO-$D_6$) δ -303.93.



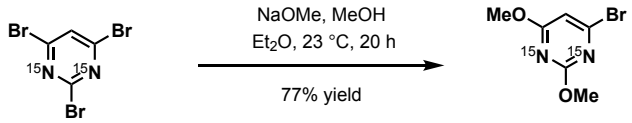

62 69

**Barbituric acid-$^{15}N_2$, 69**. To a freshly prepared solution of sodium ethoxide obtained by dissolving 0.65 g (28.4 mmol) of sodium in dry ethanol (16.5 mL) diethyl malonate (4.31 mL, 28.4 mmol) was added. The reaction mixture was stirred until a lot of white precipitate formed. To this mixture urea-$^{15}N_2$ (1.76 g, 28.4 mmol) was added and the resultant suspension was refluxed for 48 h. Next, the reaction was cooled to room temperature and acidified with 12M HCl to pH = 1. The white precipitate was filtered off and sequentially washed with ethanol (20.0 mL) and water (2×30.0 mL). The filtrate was concentrated to dryness and mixed with 20.0 mL of ethanol/water mixture (1:1, v/v) to induce precipitation of the remaining product. After the filtration and wash with 10.0 mL of ethanol/water mixture (1:1, v/v) the material was combined with previous fraction and dried under vacuum to afford 3.33 g (25.62 mmol, 90% yield) of barbituric acid-$^{15}N_2$ as a white powder. $^1$H NMR (600 MHz, DMSO-D$_6$) δ 11.10 (d, $J$ = 91.1 Hz, 2H, 2NH), 3.46 (s, 2H, CH$_2$). $^{13}$C NMR (126 MHz, DMSO-D$_6$) δ 167.83 (d, $J$ = 10.9 Hz), 151.71 (t, $J$ = 18.1 Hz), 39.52. $^{15}$N NMR (41 MHz, DMSO-D$_6$) δ -228.22. GS-MS (m/z): [M]$^+$ calcd for $C_4H_4{}^{15}N_2O_3$, 130.0163; found, 130.0158.



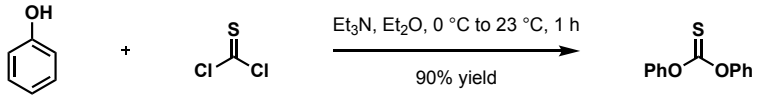

69

**2,4,6-Tribromopyrimidine-$^{15}N_2$**. The title compound was prepared according to the procedure reported by White and Hansen[34] starting from 3.40 g (26.2 mmol) of barbituric acid-$^{15}N_2$ to produce 7.90 g (24.8 mmol, 94% yield) of material as a pale yellow solid. $^1$H NMR (500 MHz, $CDCl_3$) δ 7.73 (s, 1H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 153.14 (d, $J$ = 1.7 Hz), 150.82 (t, $J$ = 3.7 Hz), 128.24 (t, $J$ = 2.5 Hz). $^{15}$N NMR (41 MHz, $CDCl_3$) δ -81.24. GS-MS (m/z): $[M]^+$ calcd for $C_4HBr_3{}^{15}N_2$, 315.7631; found, 315.7630.



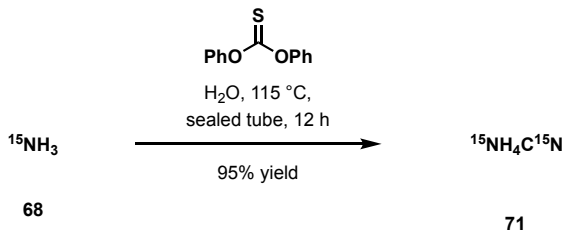

70

**4-Bromo-2,6-dimethoxypyrimidine-1,3-$^{15}N_2$, 70**. The title compound was prepared according to the procedure reported by White and Hansen[4] starting from 7.80 g (24.5 mmol) of 2,4,6-tribromopyrimidine-$^{15}N_2$ to produce 4.15 g (18.8 mmol, 77% yield) of material as a white solid. $^1$H NMR (400 MHz, $CDCl_3$) δ 6.57 (s, 1H), 3.98 (s, 3H), 3.94 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 171.74 (d, $J$ = 8.7 Hz), 164.50 (dd, $J$ = 9.1, 5.6 Hz), 152.02 (d, $J$ = 3.4 Hz), 105.09 (dd, $J$ = 2.8, 1.6 Hz), 55.51 (dd, $J$ = 3.4, 2.8 Hz), 54.49 (d, $J$ = 3.9 Hz). $^{15}$N NMR (41 MHz, $CDCl_3$) δ -145.49 (d, $J$ = 1.0 Hz), -165.63 (d, $J$ = 1.1 Hz). HRMS-EI (m/z): $[M+H]^+$ calcd for $C_6H_8Br{}^{15}N_2O_2$, 220.9710; found, 220.9715.



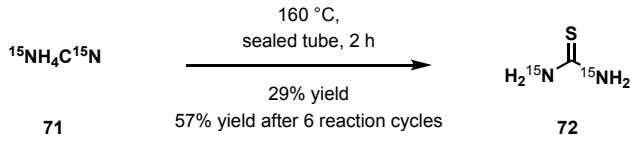

**Diphenyl thiocarbonate.** In a three neck 1 L round bottom flask equipped with a magnetic stirring bar, thermometer and drying tube 27.8 g (0.295 mol) of phenol and 82.0 mL (0.590 mol) of trimethylamine were dissolved in 600.0 mL of diethyl ether. After the reaction mixture was cooled to 0 °C, thiophosgene (11.30 mL, 0.147 mol) was added dropwise via

syringe over 30 min period inducing immediate formation of precipitate. The reaction was allowed to warm to room temperature and stirred for 1 h. Next, the solution was poured into 1.0 L of water, the organic phase was separated and the aqueous phase was additionally extracted with diethyl ether (3×100 mL). The combined organic phase was washed with water (2×500 mL), dried over $MgSO_4$ and concentrated to dryness under reduced pressure. The crude product was recrystallized from hexanes to afford 30.80 g (0.134 mol, 90% yield) of diphenyl thiocarbonate as a white crystalline solid. $^1$H NMR (500 MHz, $CDCl_3$) δ 7.48 (t, $J$ = 7.9 Hz, 4H), 7.34 (t, $J$ = 7.4 Hz, 2H), 7.24 (d, $J$ = 7.5 Hz, 4H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 194.68, 153.45, 129.56, 126.71, 121.72. HRMS-EI (m/z): $[M+H]^+$ calcd for $C_{13}H_{12}O_2S$, 232.0558; found, 232.0546.



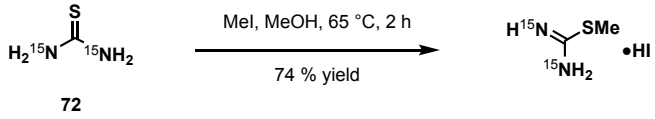

**Ammonium isothiocyanate-$^{15}N_2$, 71**. Ammonium chloride (2.50 g, 45.87 mmol, >99% $^{15}$N isotope incorporation) was mixed with 5.0 g (0.125 mol) of granular NaOH in a 25 mL one neck round bottom flask equipped with a magnetic stirring bar and an outlet for ammonia connected to a 25-mL round bottom glass pressure vessel cooled to -78 °C through the tube, filled with granular sodium hydroxide. The mixture was heated with blow torch until no more ammonia was condensing in the receiving vessel. After the weight of collected ammonia was measured (0.82 g, 45.6 mmol, 99% recovery) it was carefully mixed with water (4.0 mL) at -78°C. To the frozen mixture diphenyl

thiocarbonate (4.80 g, 20.87 mmol) was added and the vessel was sealed. After the mixture was warmed up to room temperature it was placed into an oil bath heated at 115 °C and stirred at this temperature for 12 h. The reaction was cooled to room temperature, diluted with water (30.0 mL) and phenol was removed by extraction with $Et_2O$ (3×35.0 mL). The aqueous phase was separated and concentrated to dryness under reduced pressure. The residual moisture was removed by azeotropic evaporation with methanol to produce title compound (1.70 g, 21.8 mmol, 95% yield based on $^{15}NH_4Cl$) as a white powder. $^{13}C$ NMR (101 MHz, $D_2O$) δ 133.50 (d, $J$ = 13.4 Hz). $^{15}N$ NMR (41 MHz, $D_2O$) δ -174.65.




71 72

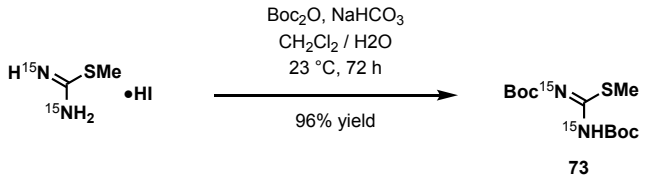
**Thiourea-$^{15}N_2$, 72**. The title compound was prepared according to the procedure reported by Fischer et al. [96] starting from 8.40 g (0.108 mol) of ammonium isothiocyanate-$^{15}N_2$ to produce 4.8 g (61.54 mmol, 57 % yield) of the title compound as pinkish solid after six reaction cycles. $^{13}C$ NMR (126 MHz, acetone-$D_6$) δ 186.20 (t, $J$ = 15.5 Hz). $^{15}N$ NMR (41 MHz, acetone-$D_6$) δ -276.45.




72

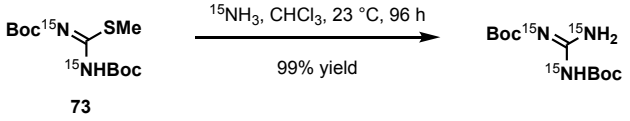
***S*-Methylisothiourea-$^{15}N_2$ hydroiodide.** A solution of thiourea-$^{15}N_2$ (4.00 g, 51.3 mmol) in 40.0 mL of methanol was added to a 100-mL round bottom flask equipped with a magnetic stirring bar and a reflux condenser. After iodomethane (3.20 mL, 51.3 mmol) was added in one portion the mixture was refluxed for 2 h. Next, the reaction mixture was allowed to cool to room temperature and concentrated to dryness under reduced pressure

providing yellowish solid that was mixed with EtOAc (50.0 mL) and filtered. The precipitate was sequentially washed several times with EtOAc and diethyl ether until it become completely colorless. The product was dried under vacuum to provide 8.35 g (38.0 mmol, 74% yield) of white powder. $^{1}$H NMR (500 MHz, DMSO-$D_6$) δ 8.81 (br. s, 4H), 2.56 (s, 3H). $^{13}$C NMR (126 MHz, DMSO-$D_6$) δ 171.06 (t, $J$ = 18.1 Hz), 13.61. $^{15}$N NMR (41 MHz, DMSO-$D_6$) δ -271.08.

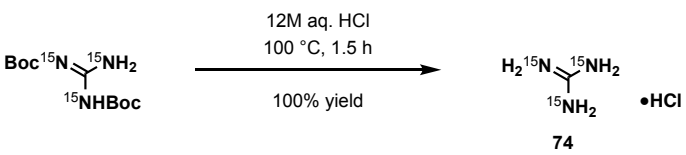



**Di-*tert*-Butyloxycarbonyl-S-methylisothiourea-$^{15}N_2$, 73.** The title compound was prepared according to the procedure reported by Hammerschmid et al. [97] with minor modifications. To a suspension of *S*-Methylisothiourea-$^{15}N_2$ hydroiodide (8.20 g, 37.3 mmol) in dichloromethane (90.0 mL) $Boc_2O$ (24.4 g, 119.9 mmol) was added in one portion followed by the addition of 90.0 mL of saturated aqueous $NaHCO_3$ solution. The resultant heterogeneous mixture was vigorously stirred at room temperature for 72 h. The layers were separated and the aqueous phase was extracted with dichloromethane (3×50 ml). The combined organic phase was dried over $Na_2SO_4$ and concentrated under reduced pressure. Excess $Boc_2O$ was removed by vacuum distillation at 0.5 mmHg and the crude material was purified by column chromatography (3% EtOAc in hexanes) to afford the desired product as a white solid (10.41 g, 35.65 mmol, 96% yield). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 11.58 (d, $J$ = 90.9 Hz, 1H), 2.36 (s, 3H), 1.49 (s, 9H), 1.47 (s, 9H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 171.51 (dd, $J$ = 12.5, 2.5 Hz), 160.87 (d, $J$ = 7.9 Hz), 150.87 (d, $J$ = 25.0 Hz), 83.34, 81.06 (d, $J$ = 1.8 Hz), 28.16, 14.51 (d, $J$ = 5.8 Hz). $^{15}$N NMR (41 MHz,

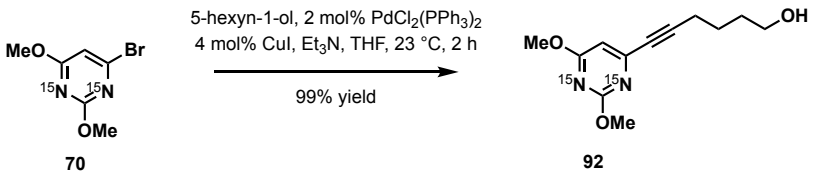
$CDCl_3$) δ -169.17 (d, $J$ = 0.9 Hz), -245.99 (d, $J$ = 1.1 Hz). HRMS-EI (m/z): $[M+Na]^+$ calcd for $C_{12}H_{22}{}^{15}N_2NaO_4S$, 315.1139; found, 315.1139.




**Di-*tert*-Butyloxycarbonylguanidine-$^{15}N_3$.** Ammonium chloride (1.55 g, 28.4 mmol, >99% $^{15}N$ isotope incorporation) was mixed with 3.0 g (0.075 mol) of granular NaOH in a 25 mL one neck round bottom flask equipped with a magnetic stirring bar and an outlet for ammonia connected to a 25-mL round bottom glass pressure vessel cooled to -78 °C through the tube, filled with granular sodium hydroxide. The mixture was heated with blow torch until no more ammonia was condensing in the receiving vessel. After the weight of collected ammonia was measured (0.50 g, 27.8 mmol, 98% recovery) it was carefully mixed with a solution of di-*tert*-Butyloxycarbonyl-S-methylisothiourea-$^{15}N_2$ (5.2 g, 17.8 mmol) in chloroform (8.0 mL) at -78°C. The reaction mixture was warmed to room temperature and stirred for 96 h. Next the mixture was filtered and concentrated to produce the desired product (4.60 g, 17.6 mmol, 99% yield) as a white solid. $^1H$ NMR (500 MHz, $CDCl_3$) δ 8.26 (s, 3H), 1.42 (s, 18H). $^{13}C$ NMR (126 MHz, $CDCl_3$) δ 158.46 (dt, $J$ = 21.1, 13.7 Hz), 81.08 (br. s), 28.13. $^{15}N$ NMR (41 MHz, cdcl$_3$) δ -293.17 (s). HRMS-EI (m/z): $[M+Na]^+$ calcd for $C_{11}H_{21}{}^{15}N_3NaO_4$, 285.1341; found, 285.1338.

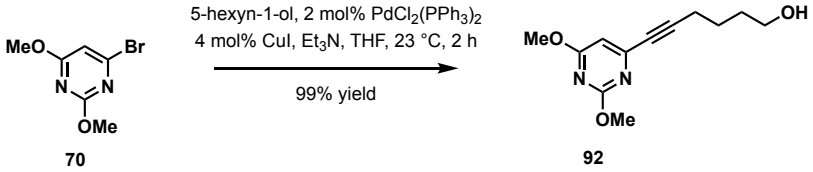


**Guanidine-$^{15}N_3$ hydrochloride, 74.** Di-*tert*-butyloxycarbonylguanidine-$^{15}N_3$ (6.1 g, 23.4 mmol) was mixed with 12M HCl and the mixture was refluxed for 1.5 h. The resultant homogenous colorless mixture was cooled to room temperature and concentrated under reduced pressure. The residual moisture was removed by azeotropic evaporation with methanol to provide guanidine-$^{15}N_3$ hydrochloride (2.30 g, 23.4 mmol, 100% yield) as a white solid. $^1$H NMR (400 MHz, DMSO-D$_6$) δ 7.20 (d, $J$ = 90.8 Hz, 1H). $^{13}$C NMR (151 MHz, DMSO-D$_6$) δ 158.37 (q, $J$ = 20.1 Hz). $^{15}$N NMR (41 MHz, DMSO-D$_6$) δ -303.29. $^{15}$N NMR (41 MHz, DMSO-D$_6$) δ -303.29.




**Compound-$^{15}N_2$, 92.** A 250 mL one neck round bottom flask equipped with a magnetic stirring bar under argon was charged with 5-hexyn-1-ol (2.7 g, 27.5 mmol), 4-bromo-2,6-dimethoxypyrimidine-1,3-$^{15}N_2$ **70** (4.05 g**,** 18.3 mmol), trimethylamine (34.0 mL) and THF (34.0 mL). The reaction mixture was cooled to -30 °C and thoroughly degassed via three vacuum and argon backfill cycles. Next, $PdCl_2(PPh_3)_2$ (0.257 g, 0.37 mmol) and CuI (0.139 g, 0.73 mmol) were added under an argon flow. The reaction mixture was allowed to warm to room temperature and stirred until the complete disappearance of the starting material was observed by TLC (2 h). The resultant black mixture was poured into 150.0 mL of water and the product was extracted with EtOAc (3×100 mL). The combined organic phase was dried over $Na_2SO_4$ and concentrated to dryness under reduced pressure. The crude product was purified by column chromatography (65% EtOAc in hexanes) to afford the title compound (4.29 g, 18.0 mmol, 98% yield) as a pale-yellow oil. $^1$H NMR

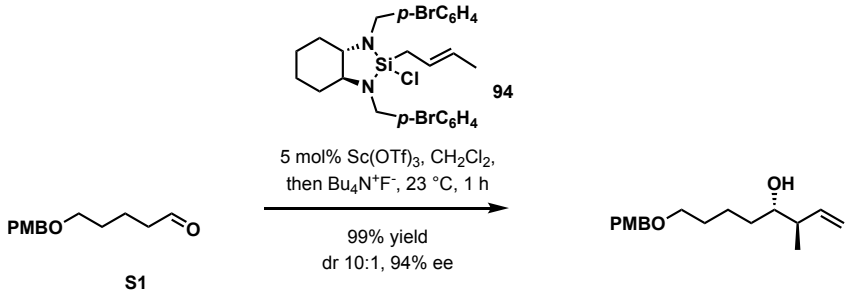
(500 MHz, $CDCl_3$) δ 6.39 (s, 1H), 3.96 (s, 3H), 3.93 (s, 3H), 3.73 – 3.59 (m, 2H), 2.62 – 2.35 (m, 2H), 1.90 (s, 1H), 1.79 – 1.50 (m, 4H). $^{13}C$ NMR (126 MHz, $CDCl_3$) δ 171.85 (d, $J$ = 9.0 Hz), 165.38 (dd, $J$ = 9.0, 6.0 Hz), 151.89 (d, $J$ = 3.8 Hz), 106.71 – 102.53 (m), 94.16 (d, $J$ = 1.9 Hz), 79.25 (d, $J$ = 10.0 Hz), 62.18, 55.43 – 54.39 (dd, $J$ = 3.31, 2.81 Hz), 54.00 (d, $J$ = 4.0 Hz), 31.90, 24.51, 19.31. $^{15}N$ NMR (41 MHz, $CDCl_3$) δ -152.69 (d, $J$ = 1.0 Hz), -163.14 (d, $J$ = 1.0 Hz). HRMS-EI (m/z): $[M+H]^+$ calcd for $C_{12}H_{17}{}^{15}N_2O_3$, 239.1180; found, 239.1180.



**70** **92**

**Compound 92.** The title compound was obtained (14.08 g, 59.6 mmol, 99% yield) using the procedure described above for its $^{15}N$-analog starting from 12.95 g (59.7 mmol) of compound **70**, 8.8 g (89.7 mmol) of 5-hexyn-1-ol, 0.84 g (1.2 mmol) of $PdCl_2(PPh_3)_2$, 0.45 g (2.37 mmol) of CuI, 109.0 mL of trimethylamine and 109.0 mL of THF. $^1H$ NMR (500 MHz, $CDCl_3$) δ 6.38 (s, 1H), 3.94 (s, 3H), 3.91 (s, 3H), 3.72 – 3.62 (m, 2H), 2.50 – 2.40 (m, 2H), 2.19 (s, 1H), 1.75 – 1.62 (m, 4H). $^{13}C$ NMR (126 MHz, $CDCl_3$) δ 171.83, 165.36, 151.87, 104.41, 94.19, 79.19, 62.10, 54.99, 54.00, 31.86, 24.48, 19.28. HRMS-EI (m/z): $[M+H]^+$ calcd for $C_{12}H_{17}N_2O_3$, 237.1239; found, 237.1247. $[M+H]^+$ calcd for $C_{12}H_{17}N_2O_3$, 237.1239; found, 237.1247.

**Optimization of the crotylation protocol with Leighton's (*S*,*S*)-crotylsilane 94.**

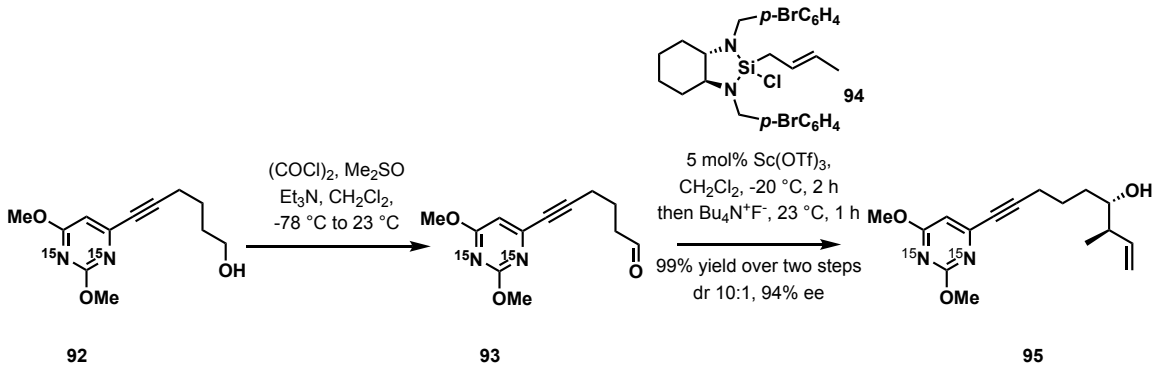



| Reaction temp., °C | Reaction time | Results |
|---|---|---|
| 0 | 15 min | 71% yield, 88% *ee*[a] |
| -20 | 20 min | 99% yield, 91% *ee*[a] |
| -40 | 2 h 45 min | 95% yield, 91% *ee*[a] |
| -78 | 7 h | 30% conversion[b], 91% *ee*[a] |

[a]measured by HPLC; [b]determined by $^1$H NMR of the crude reaction mixture

Aldehyde **S1** was prepared according to the procedure reported by Jung and co-workers.[98] The temperature effect was investigated using the following procedure. A 5.0 mL two neck round bottom flask equipped with a magnetic stirring bar, thermometer and argon inlet adapter was added aldehyde (50.0 mg, 0.224 mmol) and 2.2 mL of dichloromethane. The reaction mixture was cooled to the appropriate temperature and Leighton's (*S*,*S*)-crotylsilane[99] **94** (0.153 mg, 0.270 mmol, *E*/*Z* 10:1) was added followed by the addition of $Sc(OTf)_3$ (5.5 mg, 11 μmol). The mixture was stirred and monitored by TLC until complete disappearance of aldehyde was observed. Next, a 1M solution of tetrabutylammonium fluoride in THF (224 μL, 0.224 mmol) was added and the mixture was allowed to warm to room temperature. The solvent was removed under reduced pressure and the residue was fractionated by column chromatography (30% EtOAc in hexanes). Fractions containing the desired product were combined and the title compound was isolated in a form of colorless oil after the second column chromatography purification using the abovementioned solvent system. The enantiopurity of the material was

determined in each case by HPLC analysis. [Chiralcel ® AD-H; 1% *i*-PrOH- Hexanes; flow rate = 1 mL/ min; detection at 215 nm; $t_1$ = 50.60 min. (major), $t_2$ = 57.28 min. (minor)]. $[\alpha]_{20}^{D}$ - 1.265° (*c* 1.0, $CHCl_3$). $^1$H NMR (500 MHz, $CDCl_3$) δ 7.26 (d, J = 8.7 Hz, 2H), 6.87 (d, J = 8.6 Hz, 2H), 5.81 – 5.69 (m, 1H), 5.15 – 5.04 (m, 2H), 4.43 (s, 2H), 3.80 (s, 3H), 3.49 – 3.40 (m, 2H), 3.42 – 3.35 (m, 1H), 2.25 – 2.14 (m, 1H), 1.76 – 1.33 (m, 7H), 1.02 (d, J = 6.9 Hz, 3H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ159.07, 140.29, 130.69, 129.19, 116.16, 113.71, 74.55, 72.51, 70.01, 55.23, 44.06, 33.95, 29.70, 22.44, 16.23. HRMS-EI (m/z): $[M+H]^+$ calcd for $C_{17}H_{27}O_3$, 279.1960; found, 279.1948.



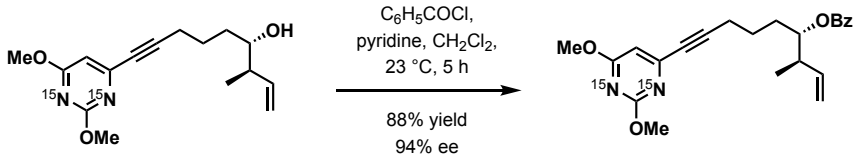

**Compound-$^{15}N_2$, 95.** A diluted solution of oxalyl chloride (2.6 mL, 36.0 mmol) in 160.0 mL of dichloromethane in a 500 mL one neck round bottom flask equipped with a magnetic stirring bar under argon was cooled to -78 °C. $Me_2SO$ (2.8 mL, 39.5 mmol) was added dropwise over 5 min period followed by stirring at -78 °C for 20 min. The solution of alcohol **92** (4.28 g, 18.0 mmol) in 20.0 mL of dichloromethane was added dropwise over 3 min. The reaction mixture was allowed to stir at -78 °C for 10 min before trimethylamine (10.0 mL, 71.9 mmol) was added in one portion. The cooling bath was removed and the reaction was allowed to slowly warm. Once full conversion was observed by TLC, the reaction was poured in water (200.0 mL). The organic phase was separated

and the aqueous phase was additionally extracted with dichloromethane (3×70 mL). The combined organic phase was washed with aqueous saturated ammonium chloride (200.0 mL), dried over $Na_2SO_4$ and concentrated to dryness under reduced pressure. The crude product was purified by column chromatography (25% EtOAc in hexanes → 50% EtOAc in hexanes) to yield a colorless oil that was immediately used for the next step due to aldehyde instability.

The aldehyde was dissolved in 180.0 mL of dichloromethane in a two neck 500.0 mL round bottom flask equipped with stirring bar, thermometer and argon inlet adapter. The mixture was cooled to -20 °C and Leighton's (*S*,*S*)-crotylsilane **94** (15.3 g, 26.9 mmol, *E*/*Z* 10:1) was added followed by the addition of $Sc(OTf)_3$ (0.44 g, 0.90 mmol) in one portion. The mixture was stirred at -20 °C until complete disappearance of aldehyde was observed by TLC (1.5 - 2 h). Next, a solution of tetrabutylammonium fluoride (9.4 g, 36.0 mmol) in 20.0 mL of dichloromethane was added and the mixture was warmed to room temperature. The solvent was removed under reduced pressure and the residue was fractionated by column chromatography (35% EtOAc in hexanes). Fractions containing the desired product were combined and the title compound (4.87 g, 16.7 mmol, 93% yield over two steps, dr 10:1) was isolated in a form of yellowish oil after the second column chromatography purification using the abovementioned solvent system. The enantiopurity of the material was determined by HPLC analysis after the derivatization with benzoyl chloride (see below).

Major isomer: $[\alpha]_{19}^{D}$ - 0.4° (*c* 1.0, $CHCl_3$). $^1$H NMR (500 MHz, $CDCl_3$) δ 6.39 (s, 1H), 5.81 – 5.65 (m, 1H), 5.18 – 5.00 (m, 2H), 3.96 (s, 3H), 3.92 (s, 3H), 3.45 – 3.34 (m, 1H), 2.45 (t, *J* = 6.9 Hz, 2H), 2.23 – 2.12 (m, 1H), 1.88 – 1.77 (m, 1H), 1.72 – 1.59 (m, 2H), 1.53

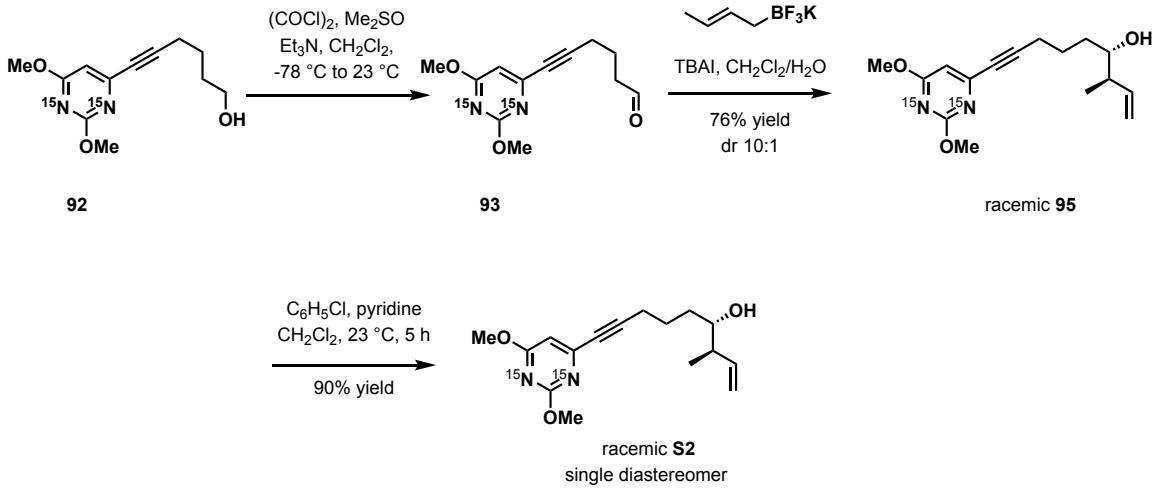
– 1.44 (m, 1H), 1.02 (d, $J$ = 6.8 Hz, 3H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 171.84 (d, $J$ = 8.7 Hz), 165.38 (dd, $J$ = 9.1, 6.1 Hz), 151.92 (d, $J$ = 3.5 Hz), 140.25, 116.62, 105.77 – 103.23 (m), 94.25 (d, $J$ = 1.9 Hz), 79.27 (d, $J$ = 10.0 Hz), 74.18 (d, $J$ = 9.6 Hz), 55.08 – 54.95 (dd, $J$ = 3.3, 2.7 Hz), 53.99 (d, $J$ = 4.0 Hz), 44.41, 33.39, 24.48, 19.51, 16.36. $^{15}$N NMR (41 MHz, $CDCl_3$) δ -152.44 (d, $J$ = 1.0 Hz), -162.95 (d, $J$ = 1.0 Hz). HRMS-EI (m/z): $[M+H]^+$ calcd for $C_{16}H_{23}{}^{15}N_2O_3$, 293.1649; found, 293.1635.




**95** **S2**

**Compound-$^{15}N_2$, S2**. A solution containing compound **95** (10.0 mg, 34.4 μmol) and pyridine (25 μL, 0.31 mmol) in dichloromethane (0.2 mL) was added to a 1 dram vial and cooled to 0 °C. After benzoyl chloride (36 μL, 0.31 mmol) was added in one portion the reaction was allowed to warm to room temperature and stirred overnight. The mixture was diluted with dichloromethane (5 mL) and washed with water (5 mL). The organic phase was dried over $Na_2SO_4$ and the solvent was removed by evaporation under reduced pressure. The crude product was purified by column chromatography (10% EtOAc in hexanes) to afford the title compound (12.0 mg, 30.0 μmol, 88% yield, single diastereomer) as yellowish oil. ee: 94% [Chiralcel ® AD-H; 2% *i*-PrOH- Hexanes; flow rate = 1 mL/ min; detection at 254 nm; $t_1$ = 14.09 min. (major), $t_2$ = 15.42 min. (minor)]. $[\alpha]_{23}^{D}$- 3.8° (*c* 0.53, $CHCl_3$). $^1$H NMR (600 MHz, $CDCl_3$) δ 8.04 (d, $J$ = 8.4 Hz, 2H), 7.56 (t, $J$ = 7.4 Hz, 1H), 7.44 (t, $J$ = 7.8 Hz, 2H), 6.41 (s, 1H), 5.93 – 5.79 (m, 1H), 5.20 – 5.13 (m, 1H), 5.13 – 5.02 (m, 2H), 3.98 (s, 3H), 3.95 (s, 3H), 2.61 – 2.51 (m, 1H), 2.49 – 2.43 (m, 2H), 1.85 – 1.79 (m, 2H), 1.75 – 1.63 (m, 2H), 1.09 (d, $J$ = 6.9 Hz, 3H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 171.95 – 171.38 (m), 166.28, 165.28, 151.87 – 151.26 (m), 139.19,

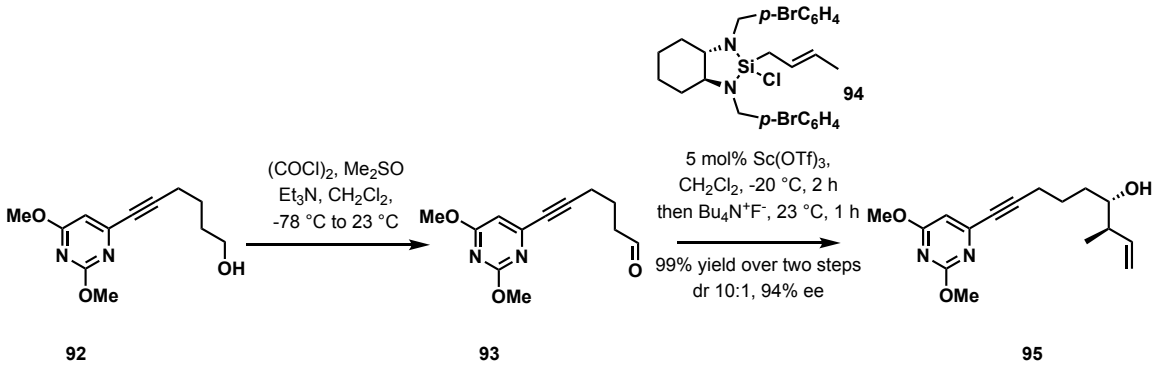
132.89, 130.43, 129.59, 128.36, 115.90, 104.51, 93.63, 79.28, 76.75, 54.93 (dd, $J$ = 3.4, 2.5 Hz), 53.91 (d, $J$ = 4.0 Hz), 41.84, 30.83, 24.21, 19.29, 16.15. $^{15}$N NMR (41 MHz, $CDCl_3$) δ -152.47, -162.82. HRMS-EI (m/z): $[M+H]^+$ calcd for $C_{23}H_{27}{}^{15}N_2O_4$, 397.1912; found, 397.1907.



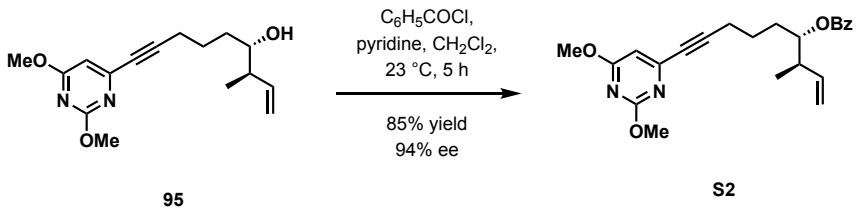

**Preparation of the racemic compound-$^{15}N_2$, S2**. The aldehyde **93** (33 mg, 0.141 mmol) was obtained according to the procedure reported above.

Aldehyde **93** was dissolved in a mixture of $CH_2Cl_2$ (1 mL) and water (1mL). To the mixture, potassium (*E*)-crotyltrifluoroborate (55 mg, 0.338 mmol, *E*/*Z* 10:1) and tetrabutylammonium iodide (5 mg, 14 μmol) were added. The reaction was vigorously stirred at ambient temperature for 30 min. The reaction mixture was diluted with $CH_2Cl_2$ (15 mL) and water (15 mL) and transferred to a separation funnel. The organic phase was separated and the aqueous solution was additionally extracted with $CH_2Cl_2$ (2×15 mL). The combined organic phase was dried over $Na_2SO_4$ and concentrated to dryness. The crude product was purified by column chromatography (35% EtOAc in hexanes) to produce racemic alcohol **95** (31 mg, 0.107 mmol, dr 10:1 76% yield) as a colorless liquid.

Alcohol **95** was submitted to the reaction with benzoyl chloride to produce benzoate ester **S2** according to the method described above.




**Compound 95.** The title compound was obtained (15.64 g, 53.9 mmol, 95% yield) using the procedure described above for its $^{15}$N-analog. The aldehyde was prepared starting from 13.4 g (56.7 mmol) of alcohol **92**, 9.7 mL (0.113 mmol) of oxalyl chloride, 8.85 mL (1.2 mmol) of $Me_2SO$, 32.0 mL of trimethylamine and 190 mL of dichloromethane. Stereoselective crotylation reaction was performed using 48.4 g (0.085 mol, *E*/*Z* 10:1) of Leighton's (*S*,*S*)-crotylsilane **94**, 1.4 g (2.84 mmol, *E*/*Z* 10:1) of $Sc(OTf)_3$ and 29.6 g (0.113 mmol) of tetrabutylammonium fluoride in 567 mL of dichloromethane. The enantiopurity of the material was determined by HPLC analysis after the derivativezation with benzoyl chloride (see below). Major isomer: $[\alpha]_{20}^{D}$ - 0.7° (*c* 1.0, $CHCl_3$). $^1$H NMR (500 MHz, $CDCl_3$) δ 6.41 (s, 1H), 5.82 – 5.68 (m, 1H), 5.18 – 5.04 (m, 2H), 3.98 (s, 3H), 3.95 (s, 3H), 3.48 – 3.39 (m, 1H), 2.48 (t, *J* = 7.0 Hz, 2H), 2.25 – 2.16 (m, 1H), 1.89 – 1.79 (m, 1H), 1.75 – 1.66 (m, 2H), 1.63 (br. s, 1H), 1.57 – 1.46 (m, 1H), 1.04 (d, *J* = 6.8 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 171.88, 165.44, 151.95, 140.27, 116.71, 104.56, 94.27, 79.29, 74.16, 55.05, 54.03, 44.46, 33.42, 24.50, 19.55, 16.40. HRMS-EI (m/z): $[M+Na]^+$ calcd for $C_{16}H_{22}N_2NaO_4$, 313.1528; found, 313.1517.




**95** **S2**

**Compound S2.** The title compound was obtained (11.5 mg, 29.2 mmol, 85% yield) using the procedure described above for its $^{15}$N-analog starting from 10.0 mg (34.4 μmol) of compound **95**, 25 μL (0.31 mmol) of pyridine and 36 μL (0.31 mmol) of benzoyl chloride in 0.2 mL of dichloromethane. ee: 94% [Chiralcel ® AD-H; 2% *i*-PrOH- Hexanes; flow rate = 1 mL/ min; detection at 254 nm; $t_1$ = 14.09 min. (major), $t_2$ = 15.42 min. (minor)]. $[\alpha]_{21}^{D}$ - 8.5° (*c* 0.93, $CHCl_3$). $^1$H NMR (600 MHz, $CDCl_3$) δ 8.04 (d, *J* = 7.8 Hz, 2H), 7.56 (t, *J* = 7.3 Hz, 1H), 7.44 (t, *J* = 7.5 Hz, 2H), 6.41 (s, 1H), 5.93 – 5.78 (m, 1H), 5.18 – 5.13 (m, 1H), 5.13 – 5.04 (m, 2H), 3.98 (s, 3H), 3.95 (s, 3H), 2.61 – 2.51 (m, 1H), 2.51 – 2.43 (m, 2H), 1.87 – 1.78 (m, 2H), 1.74 – 1.64 (m, 2H), 1.09 (d, *J* = 6.8 Hz, 3H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 171.76, 166.27, 165.31, 151.73, 139.18, 132.88, 130.42, 129.58, 128.35, 115.89, 104.50, 93.61, 79.31, 76.74, 54.92, 53.90, 41.83, 30.82, 24.20, 19.28, 16.14. HRMS-EI (m/z): $[M+Na]^+$ calcd for $C_{23}H_{26}N_2NaO_4$, 417.1790; found, 417.1791.

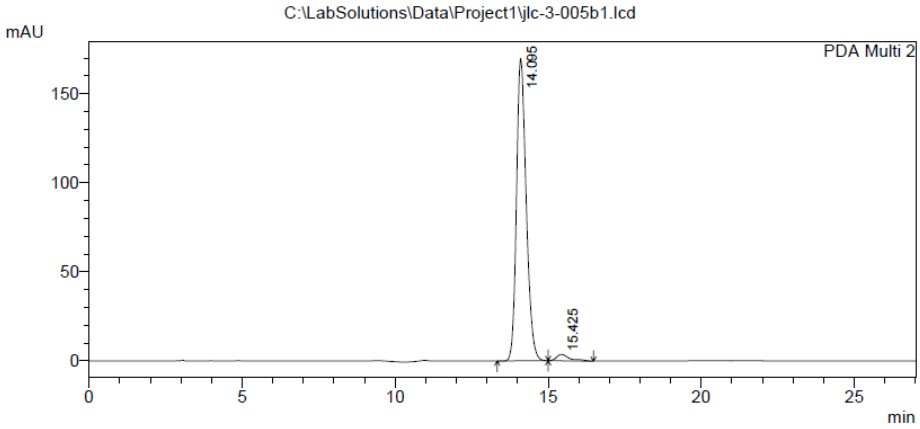
**Figure S1.** HPLC traces analysis of compound **S2** and racemic **S2** prepared via Leighton's crotylation protocol.

**==== Shimadzu LCsolution Analysis Report ====**

C:\LabSolutions\Data\Project1\jlc-3-005b1.lcd

| | |
|---|---|
| Acquired by | : Joanna Chen |
| Sample Name | : jlc-3-005b |
| Sample ID | : jlc-3-005b |
| Vail # | : |
| Injection Volume | : 10 uL |
| Data File Name | : jlc-3-005b1.lcd |
| Method File Name | : brad.lcm |
| Batch File Name | : |
| Report File Name | : Default.lcr |
| Data Acquired | : 8/14/2017 6:32:41 PM |
| Data Processed | : 8/14/2017 6:59:44 PM |

**<Chromatogram>**




1 PDA Multi 2/254nm 4nm

PeakTable

PDA Ch2 254nm 4nm

| Peak# | Ret. Time | Area | Height | Area % | Height % |
|---|---|---|---|---|---|
| 1 | 14.095 | 3744126 | 169690 | 97.158 | 97.938 |
| 2 | 15.425 | 109512 | 3573 | 2.842 | 2.062 |
| Total | | 3853637 | 173263 | 100.000 | 100.000 |

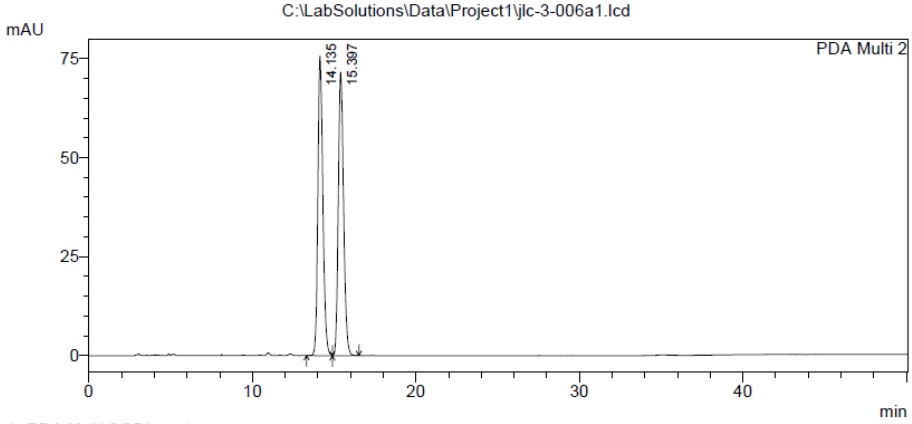
# ==== Shimadzu LCsolution Analysis Report ====

C:\LabSolutions\Data\Project1\jlc-3-006a1.lcd

| | |
|---|---|
| Acquired by | : Joanna Chen |
| Sample Name | : jlc-3-006a |
| Sample ID | : jlc-3-006a |
| Vail # | : |
| Injection Volume | : 10 uL |
| Data File Name | : jlc-3-006a1.lcd |
| Method File Name | : brad.lcm |
| Batch File Name | : |
| Report File Name | : Default.lcr |
| Data Acquired | : 8/14/2017 5:41:32 PM |
| Data Processed | : 8/14/2017 6:31:39 PM |

**<Chromatogram>**

C:\LabSolutions\Data\Project1\jlc-3-006a1.lcd

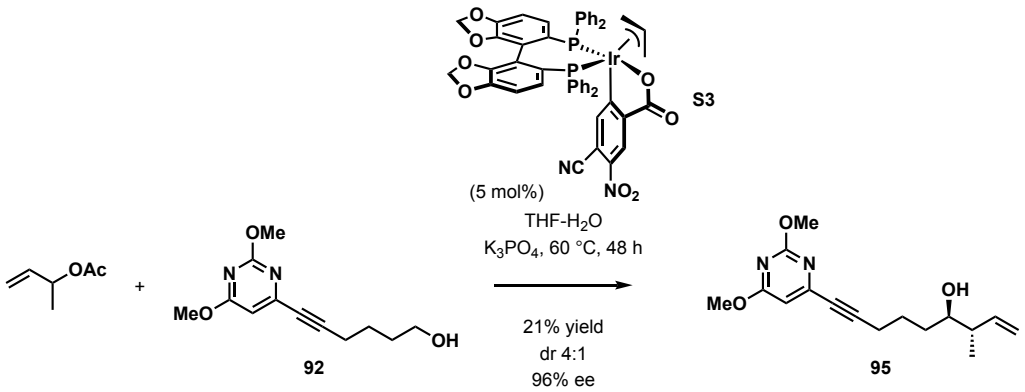



1 PDA Multi 2/254nm 4nm

PeakTable

PDA Ch2 254nm 4nm

| Peak# | Ret. Time | Area | Height | Area % | Height % |
|---|---|---|---|---|---|
| 1 | 14.135 | 1610946 | 75710 | 49.964 | 51.378 |
| 2 | 15.397 | 1613235 | 71649 | 50.036 | 48.622 |
| Total | | 3224181 | 147359 | 100.000 | 100.000 |



**Alternative procedure for synthesis of compound 95.** The method reported by Krische *et. al.*[100] was utilized in the present reaction. A flame-dried vial under an atmosphere of argon was charged with alcohol **92** (53.8 mg, 0.228 mmol), (*S*)-Iridium catalyst **S3** (12.0 mg, 11.0 μmol), potassium phosphate (24.2 mg, 0.114 mmol), THF (114 μL, 2.0M) and water (20.5 μL, 1.14 mmol). But-3-en-2-yl acetate (57.8 μL, 0.455mmol) was added and the reaction mixture was allowed to stir at ambient temperature for 30 min. Next, the reaction was heated in an oil bath at 60 °C under stirring for 48 h. The mixture was concentrated under reduced pressure and the dry residue was fractionated by column chromatography (30% EtOAc in hexanes) to afford the title compound (13.9 mg, 0.048 mmol, 21% yield, dr 4:1, 96% ee for the major diastereomer) as pale yellow oil. The enantiopurity of the material was determined by HPLC traces analysis after the derivatization with benzoyl chloride described above. $^{1}$H and $^{13}$C NMR spectra agreed with those reported above.

**Figure S2.** HPLC traces analysis of compound **S2** and racemic **S2** prepared via Krische's crotylation protocol.

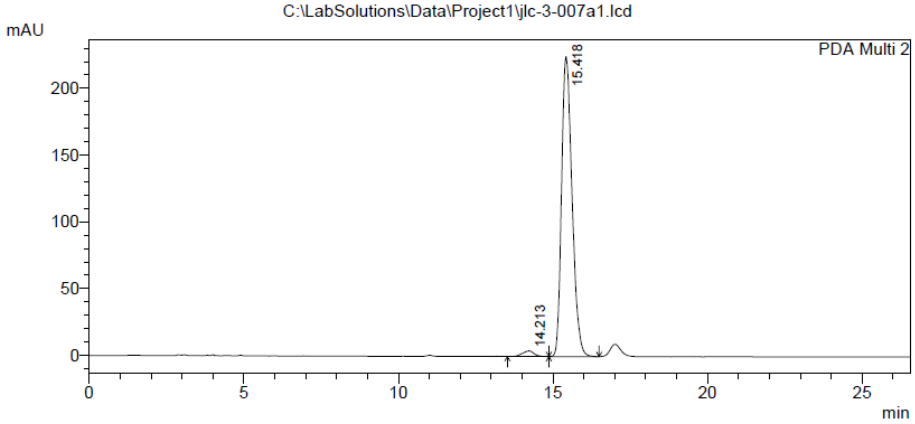
# ==== Shimadzu LCsolution Analysis Report ====

C:\LabSolutions\Data\Project1\jlc-3-007a1.lcd

| | |
|---|---|
| Acquired by | : Joanna Chen |
| Sample Name | : jlc-3-007a |
| Sample ID | : jlc-3-007a |
| Vail # | : |
| Injection Volume | : 10 uL |
| Data File Name | : jlc-3-007a1.lcd |
| Method File Name | : brad.lcm |
| Batch File Name | : |
| Report File Name | : |
| Data Acquired | : 8/15/2017 2:04:42 PM |
| Data Processed | : 8/15/2017 2:32:56 PM |

**<Chromatogram>**

C:\LabSolutions\Data\Project1\jlc-3-007a1.lcd




1 PDA Multi 2/254nm 4nm

PeakTable

PDA Ch2 254nm 4nm

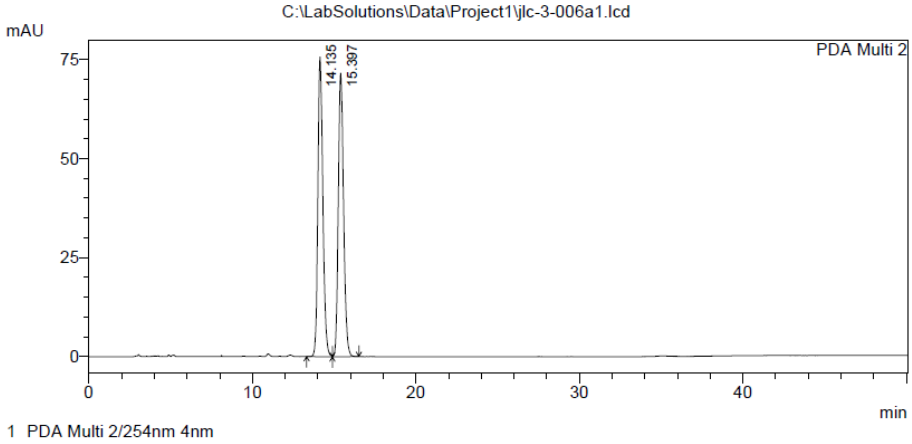
| Peak# | Ret. Time | Area | Height | Area % | Height % |
|---|---|---|---|---|---|
| 1 | 14.213 | 109731 | 4221 | 2.028 | 1.843 |
| 2 | 15.418 | 5301631 | 224760 | 97.972 | 98.157 |
| Total | | 5411362 | 228981 | 100.000 | 100.000 |

**==== Shimadzu LCsolution Analysis Report ====**

C:\LabSolutions\Data\Project1\jlc-3-006a1.lcd

| | |
|---|---|
| Acquired by | : Joanna Chen |
| Sample Name | : jlc-3-006a |
| Sample ID | : jlc-3-006a |
| Vail # | : |
| Injection Volume | : 10 uL |
| Data File Name | : jlc-3-006a1.lcd |
| Method File Name | : brad.lcm |
| Batch File Name | : |
| Report File Name | : Default.lcr |
| Data Acquired | : 8/14/2017 5:41:32 PM |
| Data Processed | : 8/14/2017 6:31:39 PM |

**<Chromatogram>**

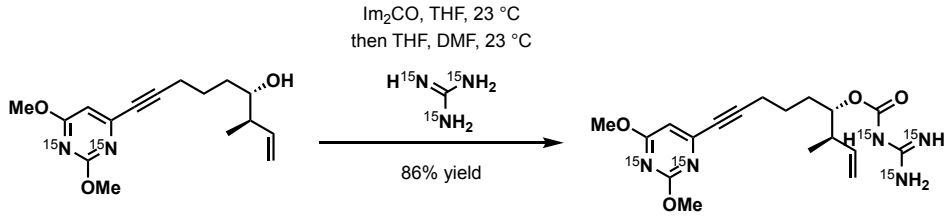



1 PDA Multi 2/254nm 4nm

PeakTable

PDA Ch2 254nm 4nm

| Peak# | Ret. Time | Area | Height | Area % | Height % |
|---|---|---|---|---|---|
| 1 | 14.135 | 1610946 | 75710 | 49.964 | 51.378 |
| 2 | 15.397 | 1613235 | 71649 | 50.036 | 48.622 |
| Total | | 3224181 | 147359 | 100.000 | 100.000 |



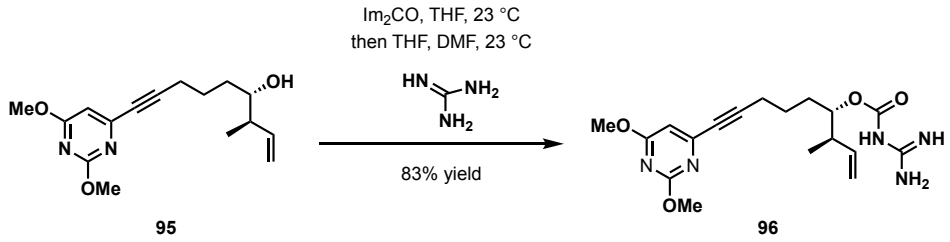

**95** **96**

**Compound-$^{15}N_5$, 96.** To a solution of alcohol **95** (5.65 g, 19.3 mmol) in THF (64.0 mL) 1,1'-carbonyldiimidazole (3.76 g, 23.2 mmol) was added and the reaction was stirred at ambient temperature overnight. The solvent was removed under reduced pressure and the

crude product was purified by column chromatography (50% EtOAc in hexanes) to produce colorless oil that was used for the next step.

To a freshly prepared solution of sodium methoxide obtained by dissolving 0.53 g (23.0 mmol) of sodium in dry methanol (116.0 mL) guanidine-$^{15}N_3$ hydrochloride (2.28 g, 23.0 mmol) was added. The reaction was stirred for 10 min and concentrated to dryness under reduced pressure. The residual methanol was removed by repetitive addition of THF and evaporation using rotary evaporator. The material was dried under vacuum for 30 min and dissolved in 97.0 mL of DMF. *N*-Acyl imidazole, obtained in the first step, was dissolved in 97.0 mL of THF and was added dropwise over 1 h period to a solution of guanidine in DMF. The reaction was stirred for an additional 10 min and poured 300.0 mL of water. The product was extracted with EtOAc (4×80 mL). The combined organic phase was sequentially washed with water (2×150 mL), saturated aqueous ammonium chloride (2×150 mL) and brine (150 mL), then dried over $Na_2SO_4$ and the organic solvent was removed under reduced pressure. The crude product was subjected to column chromatography (2% MeOH in dichloromethane → 10% MeOH in dichloromethane) to deliver the title compound (6.3 g, 16.6 mmol, 86% yield over two steps) as a white foam. $[\alpha]_{21}^{D}$ + 104.8° (*c* 1.0, $CHCl_3$). $^1$H NMR (500 MHz, $CDCl_3$) δ 7.10 (br. s, 4H), 6.38 (s, 1H), 5.79 – 5.66 (m, 1H), 5.10 – 4.93 (m, 2H), 4.79 – 4.66 (m, 1H), 3.94 (s, 3H), 3.92 (s, 3H), 2.53 – 2.32 (m, 3H), 1.75 – 1.52 (m, 4H), 0.97 (d, *J* = 6.9 Hz, 3H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 171.90 (d, *J* = 8.8 Hz), 165.20 (dd, *J* = 8.5, 7.2 Hz), 162.17 – 161.70 (m), 161.63 (d, *J* = 10.8 Hz), 151.84 (d, *J* = 4.1 Hz), 139.54, 115.52, 104.33, 94.65 (d, *J* = 1.9 Hz), 79.02 (d, *J* = 9.7 Hz), 76.82, 55.01 (t, *J* = 2.9 Hz), 54.07 (d, *J* = 3.9 Hz), 41.77, 30.32,

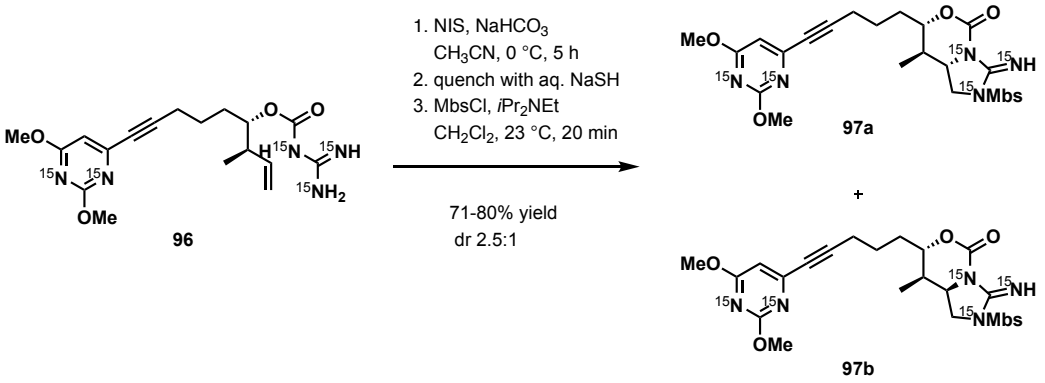
24.39, 19.26, 15.47. $^{15}$N NMR (41 MHz, $CDCl_3$) δ -152.92 (d, $J$ = 1.0 Hz), -163.97 (d, $J$ = 1.0 Hz), -237.39, -298.82 ($2^{15}$N). HRMS-EI (m/z): $[M+H]^+$ calcd for $C_{18}H_{26}{}^{15}N_5O_4$, 381.1837; found, 381.1823.




**Compound 96.** To a solution of alcohol **95** (10.0 g, 34.4 mmol) in THF (115.0 mL) 1,1'-carbonyldiimidazole (6.7 g, 41.4 mmol) was added and the reaction was stirred at ambient temperature overnight to prepare corresponding *N*-Acyl imidazole derivative.

Next, to a freshly prepared solution of sodium methoxide obtained by dissolving 3.17 g (0.138 mol) of sodium in dry methanol (200.0 mL) guanidine hydrochloride (13.2 g, 0.138 mol) was added. The reaction was stirred for 10 min and concentrated to dryness under reduced pressure. The material was dried under vacuum for 10 min and dissolved in 115.0 mL of DMF. A crude solution of *N*-Acyl imidazole, obtained in the first step, was added dropwise over 10 min period to a solution of free guanidine in DMF. The reaction was stirred for an additional 10 min and poured 600.0 mL of water. The product was extracted with EtOAc (4×150 mL). The combined organic phase was sequentially washed with water (2×400 mL), saturated aqueous ammonium chloride (2×400 mL) and brine (400 mL), then dried over $Na_2SO_4$ and the organic solvent was removed under reduced pressure. The crude product was subjected to column chromatography (2% MeOH in dichloromethane → 10% MeOH in dichloromethane) to deliver the title compound (10.76 g, 28.7 mmol, 83% yield) as a white foam. $[\alpha]_{18}^{D}$ + 103.2° (*c* 1.0, $CHCl_3$). $^1$H NMR (600 MHz, $CDCl_3$) δ 7.42 (br. s,

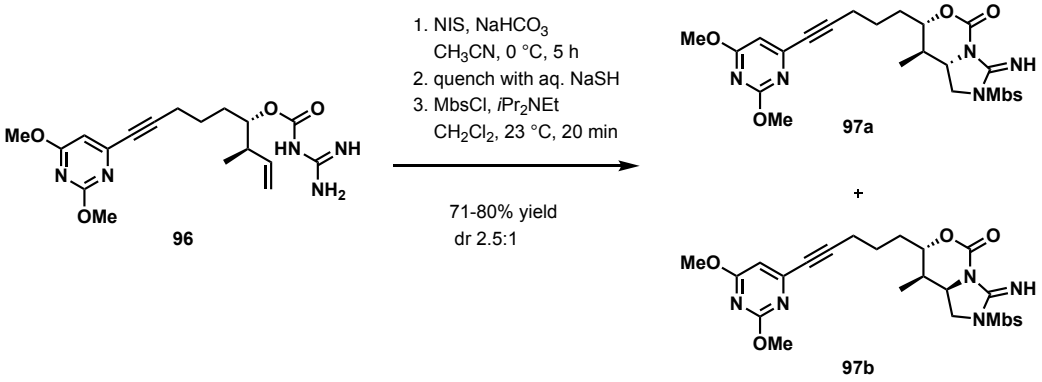
4H), 6.41 (s, 1H), 5.83 – 5.68 (m, 1H), 5.06 – 4.99 (m, 2H), 4.81 – 4.72 (m, 1H), 3.97 (s, 3H), 3.95 (s, 3H), 2.56 – 2.31 (m, 3H), 1.75 – 1.54 (m, 4H), 1.00 (d, $J$ = 6.9 Hz, 3H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 171.99, 165.29, 161.60, 161.16, 151.91, 139.52, 115.66, 104.40, 94.69, 79.11, 77.16, 55.09, 54.15, 41.86, 30.32, 24.46, 19.33, 15.51. HRMS-EI (m/z): $[M+H]^+$ calcd for $C_{18}H_{26}N_5O_4$, 376.1985; found, 376.1978.




**Stereoselective diamination of compound-$^{15}N_5$, 96.** A solution of guanidine **96** (6.3 g, 16.6 mmol) in 330.0 mL of acetonitrile was added to a 1 L round bottom flask equipped with a magnetic stirring bar and an argon inlet adapter. Sodium bicarbonate (13.9 g, 0.165 mol) was added and the mixture was cooled to 0 °C before freshly recrystallized N-iodosuccinimide (7.83 g, 34.8 mmol) was added in one portion. The heterogeneous mixture was vigorously stirred for 5 h followed by quench with 30% aqueous sodium hydrosulfide solution (300.0 mL). The crude product was extracted with EtOAc (4×100 mL). The combined organic phase was dried over $Na_2SO_4$ and concentrated to dryness under reduced pressure. The residue was dissolved in dichloromethane (165.0 mL), $i$Pr$_2$NEt (5.76 mL, 33.12 mmol) and MbsCl (4.1 g, 19.90 mmol) were added sequentially and the mixture was stirred for 30 min at ambient temperature. Next, the solution was concentrated under reduced pressure, the crude product was dissolved in EtOAc (150 mL)

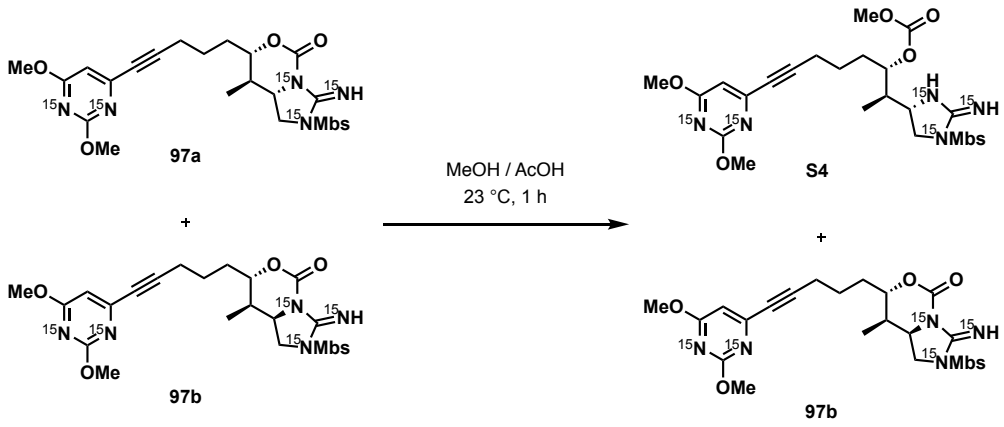
and washed with water (2×100 mL). The organic phase was separated, dried over $Na_2SO_4$ and the solvent was removed by evaporation under reduced pressure. The product was purified by column chromatography (dichloromethane → 50% EtOAc in dichloromethane) to provide the title compound (6.41 g, 11.7 mmol, 71% yield, dr 2.5:1) as a yellowish foam.

Mixture of diastereomers: $^1$H NMR (500 MHz, $CDCl_3$) δ 8.29 (br. d, $J$ = 43.4 Hz), 8.02 (d, $J$ = 9.0 Hz), 6.98 (d, $J$ = 9.0 Hz), 6.40 (s), 6.38 (s), 4.33 – 4.28 (m), 4.26 – 4.20 (m), 4.16 – 4.03 (m), 3.97 (s), 3.96 (d, $J$ = 4.1 Hz), 3.95 (s), 3.94 (s), 3.87 (s), 3.86 (s), 3.82 – 3.73 (m), 3.47 – 3.38 (m), 3.26 – 3.18 (m), 2.56 – 2.42 (m), 2.24 – 2.16 (m), 2.01 – 1.61 (m), 1.04 (d, $J$ = 6.6 Hz), 1.00 (d, $J$ = 7.1 Hz). $^{15}$N NMR (41 MHz, $CDCl_3$) δ -151.67 (d, $J$ = 0.8 Hz), -162.82 (d, $J$ = 0.8 Hz), -252.37 (dd, $J$ = 4.7, 4.8 Hz), -252.86 (dd, $J$ = 4.7, 4.8 Hz), -253.18 (dd, $J$ = 4.7, 4.8 Hz), -261.43 (dd, $J$ = 4.8, 1.5 Hz), -266.48 (dd, $J$ = 4.8, 1.6 Hz).




**Stereoselective diamination of compound 96.** This material was obtained (11.6 g, 21.3 mmol, 71% yield) using the procedure described above for its $^{15}$N-analog starting from 11.3 g (30.1 mmol) of compound **97**, 14.22 g (63.2 mmol) of N-iodosuccinimide, 25.2 g (0.3 mol) of sodium bicarbonate, 600.0 mL of acetonitrile, 7.44 g (36.1 mmol) of MbsCl, 10.5 ml (60.4 mmol) of $i$Pr$_2$NEt and 300.0 mL of dichloromethane. $^1$H NMR (600 MHz,

$CDCl_3$) δ 8.27 (br. s), 8.00 (d, *J* = 8.9 Hz), 6.96 (d, *J* = 8.9 Hz), 6.38 (s), 6.37 (s), 4.34 – 4.26 (m), 4.27 – 4.17 (m), 4.14 – 4.08 (m), 4.05 (s), 3.95 (s), 3.94 (s), 3.93 (s), 3.92 (s), 3.85 (s), 3.84 (s), 3.7 – 3.73 (m), 3.41 (t, *J* = 9.7 Hz), 3.20 (t, *J* = 9.7 Hz), 2.56 – 2.36 (m), 2.25 – 2.16 (m), 1.99 – 1.61 (m), 1.02 (d, *J* = 6.6 Hz, 3H), 0.98 (d, *J* = 7.1 Hz, 1H).



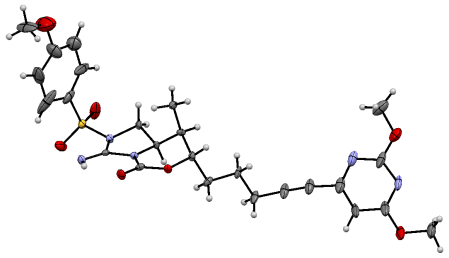

**Kinetic resolution of $^{15}$N-diastereomers 97a and 97b.** A mixture of diastereomers **97a** and **97b** (7.0 g, 12.8 mmol, dr 2.5:1) obtained in the previous step was dissolved in methanol (140.0 mL) and acetic acid (7.0 mL) and the reaction was stirred for 1 h at ambient temperature. Next, the obtained suspension was concentrated to dryness under reduced pressure and dissolved in dichloromethane (150 mL). This solution was sequentially washed with water (100 mL) and saturated aqueous sodium bicarbonate (100 mL), dried over $Na_2SO_4$ and concentrated under reduced pressure. The crude residue was fractionated by column chromatography (50% EtOAc in dichloromethane for the unreacted diastereomer **97b** → 5% MeOH in dichloromethane for the product of methanol addition **S4**) to produce 5.92 g of the product of methanol **S4** addition along with 1.72 g of recovered unreacted compound **97b**.

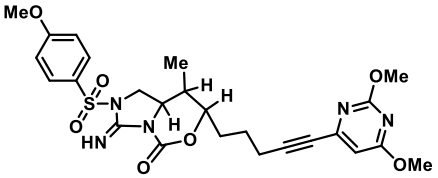
**Compound-$^{15}N_5$, S4.**

$[\alpha]_{20}^{D}$- 23.1° (*c* 1.0, $CHCl_3$). $^1$H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, *J* = 9.0 Hz, 2H), 6.93 (d, *J* = 9.0 Hz, 2H), 6.34 (s, 1H), 6.04 (br. s, 2H), 4.76 – 4.66 (m, 1H), 3.89 (s, 3H), 3.86 (s, 3H), 3.79 (s, 3H), 3.69 (s, 3H), 3.66 – 3.55 (m, 2H), 3.25 (dd, *J* = 9.1, 6.7 Hz, 1H), 2.43 – 2.32 (m, 2H), 1.78 – 1.50 (m, 5H), 0.61 (d, *J* = 7.0 Hz, 3H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 171.61 (d, *J* = 8.7 Hz), 165.15 (dd, *J* = 9.1, 6.1 Hz), 163.62, 155.47, 151.82 (dt, *J* = 22.3, 7.3 Hz), 151.57 (d, *J* = 3.7 Hz), 129.77, 128.09 (d, *J* = 3.3 Hz), 114.43, 104.34, 93.29 (d, *J* = 1.8 Hz), 79.29 (d, *J* = 10.0 Hz), 79.01, 61.03, 55.57, 54.78, 54.63, 53.79 (d, *J* = 3.9 Hz), 50.21 (d, *J* = 7.2 Hz), 41.51 (d, *J* = 1.9 Hz), 29.15, 24.02, 19.17, 10.09. $^{15}$N NMR (41 MHz, $CDCl_3$) δ -151.99 (d, *J* = 1.0 Hz), -162.77 (d, *J* = 1.0 Hz), -202.71, -245.72, -319.41. HRMS-EI (m/z): $[M+H]^+$ calcd for $C_{26}H_{34}{}^{15}N_5O_8S$, 581.1980; found, 581.1970.

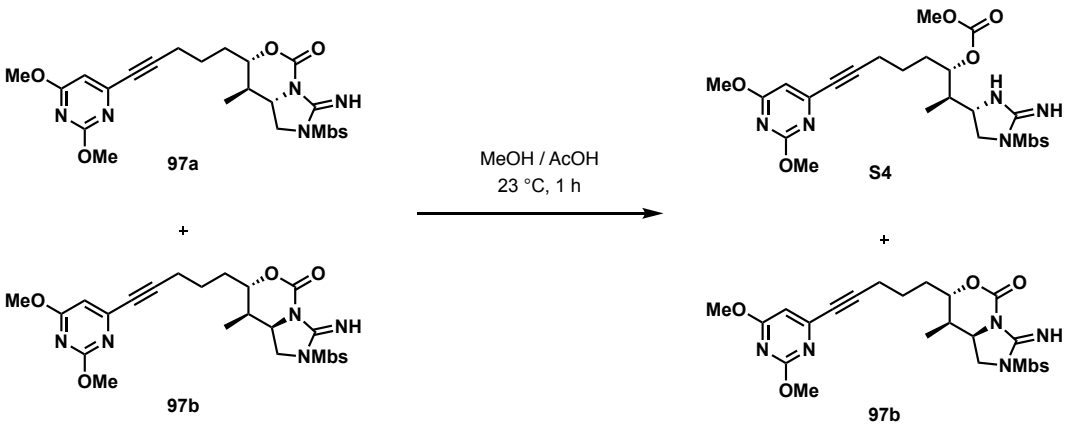
**Compound-$^{15}N_5$, 97b.**

$[\alpha]_{20}^{D}$+ 25.2° (*c* 1.0, $CHCl_3$). $^1$H NMR (500 MHz, $CDCl_3$) δ 8.29 (br. d, *J* = 57.0 Hz, 1H), 8.02 (d, *J* = 9.0 Hz, 2H), 6.98 (d, *J* = 9.0 Hz, 2H), 6.40 (s, 1H), 4.34 – 4.22 (m, 2H), 4.12 – 4.03 (m, 1H), 3.97 (s, 3H), 3.95 (s, 3H), 3.87 (s, 3H), 3.50 – 3.36 (m, 1H), 2.61 – 2.41 (m, 2H), 2.28 – 2.15 (m, 1H), 1.97 – 1.85 (m, 2H), 1.82 – 1.74 (m, 2H), 1.01 (d, *J* = 7.1 Hz, 3H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 171.75 (d, *J* = 8.7 Hz), 165.30 (dd, *J* = 8.9, 6.3 Hz), 164.00, 151.48 (d, *J* = 3.8 Hz), 148.80 (d, *J* = 22.6 Hz), 146.01 (ddd, *J* = 19.1, 14.9, 8.6 Hz), 130.93, 128.40 (d, *J* = 4.2 Hz), 114.03, 104.40 (dd, *J* = 1.8, 1.7 Hz), 92.49 (d, *J* = 1.9 Hz), 84.44, 79.80 (d, *J* = 10.0 Hz), 55.67, 54.91 (dd, *J* = 2.9, 2.8 Hz), 53.95 (d, *J* = 3.9 Hz), 51.13 (d, *J* = 7.1 Hz), 45.79 (d, *J* = 6.9 Hz), 33.57, 30.40, 23.92, 18.88, 12.11. $^{15}$N NMR (41 MHz, $CDCl_3$) δ -151.80 (d, *J* = 1.0 Hz), -162.73 (d, *J* = 1.0 Hz), -198.02 (dd, *J* = 4.6, 1.7 Hz), -253.28 (dd, *J* = 4.8, 4.7 Hz), -266.61 (dd, *J* = 4.8, 1.7 Hz). HRMS-EI (m/z): $[M+Na]^+$ calcd for $C_{25}H_{29}{}^{15}N_5NaO_7S$, 571.1537; found, 571.1552.






12b




**Kinetic resolution of $^{14}$N-diastereomers 97a and 97b.** This reaction was performed using the procedure described above starting from 12.9 g (23.7 mmol) of compound **97**, 225.0 mL of MeOH and 12.8 mL of acetic acid. The crude residue was fractionated by column chromatography to produce 10.5 g of the product of methanol addition **S4** was isolated along with 2.56 g of recovered unreacted compound **97b.**

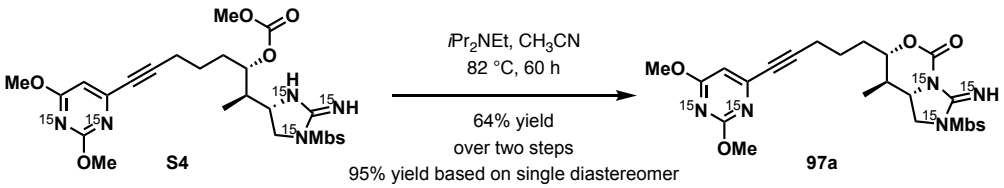
**Compound S4.**

$[\alpha]_{21}^{D}$ - 23.1° (*c* 1.0, $CHCl_3$). $^1$H NMR (500 MHz, $CDCl_3$) δ 7.83 (d, *J* = 9.0 Hz, 2H), 6.97 (d, *J* = 9.0 Hz, 2H), 6.39 (s, 1H), 5.37 (br. s, 2H), 4.78 – 4.70 (m), 3.94 (s, 3H), 3.92 (s,

3H), 3.84 (s, 3H), 3.74 (s, 3H), 3.72 – 3.66 (m, 1H), 3.66 – 3.58 (m, 1H), 3.31 (dd, $J$ = 9.4, 6.8 Hz, 1H), 2.40 (t, $J$ = 6.4 Hz, 2H), 1.81 – 1.54 (m, 5H), 0.65 (d, $J$ = 7.0 Hz, 3H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 171.64, 165.19, 163.64, 155.46), 151.51, 151.57, 129.79, 128.06, 114.43, 104.38, 93.32, 79.25, 79.04, 61.06, 55.58, 54.81, 54.63, 53.80, 50.19, 41.36, 29.30, 23.95, 19.18, 10.14. HRMS-EI (m/z): $[M+H]^+$ calcd for $C_{26}H_{34}N_5O_8S$, 576.2128; found, 576.2131.

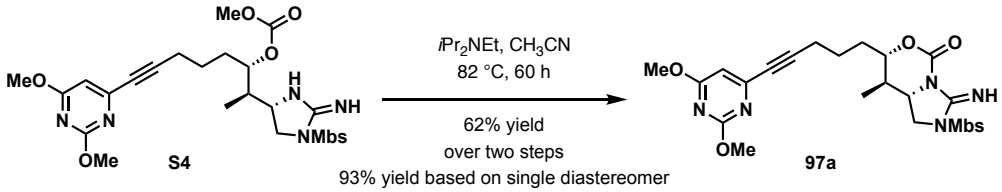
**Compound 97b.**

$[\alpha]_{22}^{D}$ + 24.4° ($c$ 1.0, $CHCl_3$). $^1$H NMR (500 MHz, $CDCl_3$) δ 8.30 (br. s, 1H), 8.03 (d, $J$ = 8.9 Hz, 2H), 6.99 (d, $J$ = 9.0 Hz, 2H), 6.41 (s, 1H), 4.35 – 4.23 (m, 2H), 4.08 (dd, $J$ = 9.1, 7.5 Hz, 1H), 3.98 (s, 3H), 3.96 (s, 3H), 3.88 (s, 3H), 3.48 – 3.41 (m, 1H), 2.62 – 2.44 (m, 2H), 2.24 – 2.17 (m, 1H), 1.97 – 1.84 (m, 2H), 1.83 – 1.66 (m, 2H), 1.02 (d, $J$ = 7.1 Hz, 3H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 171.68, 165.26, 163.92, 151.40, 148.69, 145.89, 130.88, 128.36, 113.94, 104.37, 92.34, 84.30, 79.76, 55.60, 54.84, 53.87, 51.08, 45.71, 33.52, 30.35, 23.85, 18.82, 12.07. HRMS-EI (m/z): $[M+Na]^+$ calcd for $C_{25}H_{29}N_5NaO_7S$, 566.1685; found, 566.1672.



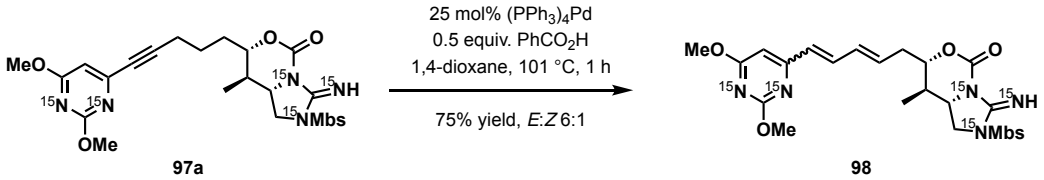

**Compound-$^{15}N_5$ 97a.** A solution of the methyl carbonate **S4** (5.92 g, 10.4 mmol) in acetonitrile (100.0 mL) containing $i$Pr$_2$NEt (18.2 mL, 0.104 mol) was refluxed for 60 h under an atmosphere of argon. Next, the reaction mixture was cooled to room temperature and the solvent was evaporated until dryness. The crude product was purified by column

chromatography (50% EtOAc in dichloromethane) to produce the title compound (4.45 g, 8.11 mmol, 64% yield over two steps, 95% yield over two steps based on single diastereomer) as a white foam. $[\alpha]_{20}^{D}$ - 21.9° (*c* 1.0, $CHCl_3$). $^1$H NMR (600 MHz, $CDCl_3$) δ 8.16 (br. d, *J* = 60.4 Hz, 1H), 7.91 (d, *J* = 8.8 Hz, 2H), 6.88 (d, *J* = 9.0 Hz, 2H), 6.29 (s, 1H), 4.14 – 4.08 (m, 1H), 4.08 – 4.02 (m, 1H), 3.86 (s, 3H), 3.84 (s, 3H), 3.76 (s, 3H), 3.74 – 3.66 (m, 1H), 3.13 (m, 1H), 2.43 – 2.32 (m, 2H), 1.89 – 1.80 (m, 1H), 1.80 – 1.69 (m, 1H), 1.68 – 1.51 (m, 3H), 0.94 (d, *J* = 6.6 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 171.59 (d, *J* = 8.7 Hz), 165.14 (dd, *J* = 8.9, 6.3 Hz), 163.87, 151.43 (d, *J* = 3.6 Hz), 149.06, 146.13 (ddd, *J* = 18.9, 15.0, 8.6 Hz), 130.89, 128.05, 113.89, 104.21, 92.74 (d, *J* = 1.9 Hz), 83.22, 79.54 (d, *J* = 10.0 Hz), 56.40 (d, *J* = 7.3 Hz), 55.58, 54.76 (dd, *J* = 3.0, 2.9 Hz), 53.80 (d, *J* = 4.0 Hz), 48.85 (d, *J* = 6.7 Hz), 35.60, 30.78, 22.47, 18.80, 11.76 (d, *J* = 1.8 Hz). $^{15}$N NMR (41 MHz, $CDCl_3$) δ -151.64 (d, *J* = 0.8 Hz), -162.74 (d, *J* = 1.0 Hz), -197.48 (dd, *J* = 4.8, 4.5 Hz), -252.43 (dd, *J* = 4.6, 4.8 Hz), -261.48 (dd, *J* = 4.8, 1.5 Hz). HRMS-EI (m/z): $[M+Na]^+$ calcd for $C_{21}H_{24}N_3O_4S$, 571.1537; found, 571.1522.



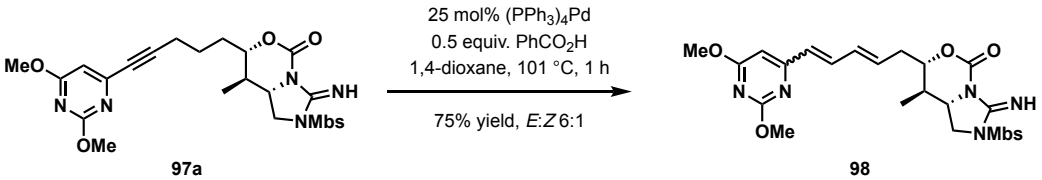

**Compound 97a.** This material was obtained (8.04 g, 14.8 mmol, 62% yield over two steps, 95% yield based on single diastereomer) using the procedure described above for its $^{15}$N-analog starting from 10.5 g (18.24 mmol) of compound **S4**, 31.6 mL (0.182 mol) of *i*$Pr_2$NEt and 182.0 mL of acetonitrile. $[\alpha]_{19}^{D}$ - 21.5° (*c* 1.0, $CHCl_3$). $^1$H NMR (600 MHz, $CDCl_3$) δ 8.31 (s, 1H), 8.04 (d, *J* = 8.9 Hz, 2H), 6.99 (d, *J* = 8.9 Hz, 2H), 6.39 (s, 1H), 4.29

– 4.20 (m, 1H), 4.18 – 4.08 (m, 1H), 3.97 (s, 3H), 3.95 (s, 3H), 3.87 (s, 3H), 3.83 – 3.72 (m, 1H), 3.26 – 3.19 (m, 1H), 2.55 – 2.43 (m, 2H), 2.00 – 1.93 (m, 1H), 1.92 – 1.82 (m, 1H), 1.79 – 1.65 (m, 3H), 1.05 (d, $J$ = 6.6 Hz, 3H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 171.59, 165.15, 163.86, 151.43, 149.00, 146.16, 130.88, 128.04, 113.88, 104.19, 92.74, 83.22, 79.53, 56.40, 55.57, 54.74, 53.79, 48.86, 35.58, 30.77, 22.46, 18.79, 11.74. HRMS-EI (m/z): $[M+H]^+$ calcd for $C_{25}H_{30}N_5O_7S$, 544.1866; found, 544.1844.




**Compound-$^{15}N_5$, 98 (mixture of E/Z isomers).** A solution of compound **97a** (4.1 g, 7.47 mmol) in 1,4-dioxane (75.0 mL) was added to a 250 mL one neck round bottom flask equipped with a magnetic stirring bar and a reflux condenser with attached argon inlet adapter. The air atmosphere was replaced with argon by 3 repeated vacuum and argon backfill cycles. To this solution, $Pd(PPh_3)_4$ (2.16 g, 1.87 mmol) and benzoic acid (0.45 g, 3.67 mmol) were added and the mixture was refluxed for 1 h. The resultant brown solution was cooled to room temperature, diluted with EtOAc (100.0 mL) and a solution of sodium diethyldithiocarbamate (0.84 g, 3.74 mmol) in water (20.0 mL) was added. The resultant biphasic mixture was stirred for 30 min, filtered through a pad of celite and the organic phase was separated. The aqueous phase was additionally extracted with EtOAc (3×70 mL) and all organic fractions were combined. After drying over $Na_2SO_4$ the solvent was removed under reduced pressure and the crude product was fractionated by column chromatography (35% EtOAc in dichloromethane) to provide the title compound (3.04 g, 5.54 mmol, 74% yield) as a yellowish foam.

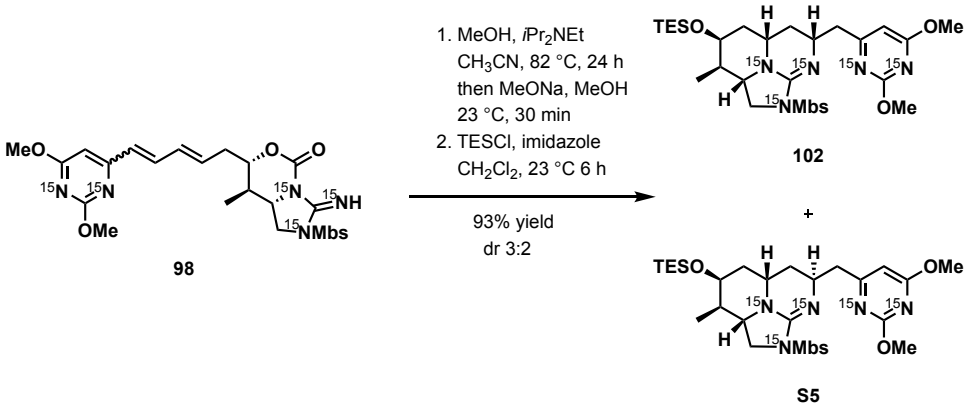
Major isomer: $^{1}$H NMR (600 MHz, $CDCl_3$) δ 8.27 (ddd, *J* = 61.7, 6.7, 3.3 Hz, 1H), 8.01 (d, *J* = 8.7 Hz, 2H), 7.37 (dd, *J* = 15.1, 11.0 Hz, 1H), 6.96 (d, *J* = 8.9 Hz, 2H), 6.35 – 6.26 (m, 2H), 6.18 (s, 1H), 6.02 – 5.93 (m, 1H), 4.25 – 4.15 (m, 2H), 3.97 (s, 3H), 3.93 (s, 3H), 3.84 (s, 3H), 3.80 – 3.69 (m, 1H), 3.24 – 3.14 (m, 1H), 2.74 – 2.63 (m, 1H), 2.47 – 2.37 (m, 1H), 1.76 – 1.65 (m, 1H), 1.01 (d, *J* = 6.6 Hz, 3H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 172.16 (d, *J* = 8.7 Hz), 165.25 – 164.60 (m), 163.87, 163.70, 148.94 (d, *J* = 22.4 Hz), 146.06 (ddd, *J* = 18.9, 14.9, 8.5 Hz), 135.44 (d, *J* = 3.6 Hz), 133.44, 131.67, 130.87, 128.80, 127.99, 113.91, 99.26, 82.84, 56.20 (d, *J* = 7.4 Hz), 55.56 (d, *J* = 3.1 Hz), 54.69 – 54.27, 53.67 (dd, *J* = 4.3, 4.2 Hz), 48.81 (d, *J* = 6.7 Hz), 34.72 (d, *J* = 9.6 Hz), 11.45 (d, *J* = 1.8 Hz). $^{15}$N NMR (41 MHz, $CDCl_3$) δ -162.05 (d, *J* = 1.0 Hz), -164.55 (d, *J* = 1.0 Hz), -197.25 (d, *J* = 2.8 Hz), -252.46 (m), -261.54 (d, *J* = 4.8 Hz). HRMS-EI (m/z): $[M+Na]^+$ calcd for $C_{25}H_{29}{}^{15}N_5NaO_7S$, 571.1537; found, 571.1522.




**Compound 98.** This material was obtained (2.43 g, 4.47 mmol, 72% yield) using the procedure described above for its $^{15}$N-analog starting from 3.0 g (5.52 mmol) of compound **97a**, 1.6 g (1.38 mol) of $Pd(PPh_3)_4$, 0.34 g (2.79 mmol) of benzoic acid and 55 ml of 1,4-dioxane.

Major isomer: $^{1}$H NMR (600 MHz, $CDCl_3$) δ 8.27 (s, 1H), 8.00 (d, *J* = 8.9 Hz, 2H), 7.37 (dd, *J* = 15.1, 11.0 Hz, 1H), 6.95 (d, *J* = 8.9 Hz, 2H), 6.34 – 6.26 (m, 2H), 6.18 (s, 1H), 6.00 – 5.94 (m, 1H), 4.25 – 4.14 (m, 2H), 3.97 (s, 3H), 3.93 (s, 3H), 3.83 (s, 3H), 3.81 – 3.74 (m, 1H), 3.20 – 3.14 (m, 1H), 2.72 – 2.65 (m, 1H), 2.46 – 2.37 (m, 1H), 1.76 – 1.66

(m, 1H), 1.01 (d, $J$ = 6.6 Hz, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 172.42, 165.19, 164.11, 163.87, 149.03, 146.09, 135.56, 133.78, 131.59, 131.14, 129.12, 114.12, 99.56, 83.00, 56.53, 55.75, 54.68, 53.89, 49.02, 35.01, 34.90, 11.71. HRMS-EI (m/z): $[M+Na]^+$ calcd for $C_{25}H_{29}N_5NaO_7S$, 566.1685; found, 566.1672.




**The assembly of $^{15}N_5$-cylindrospermopsin core by means of double Michael addition.** A mixture of 1,3-diene **98** (3.0 g, 5.47 mmol), MeOH (8.85 mL, 0.219 mol) and $i$Pr$_2$NEt (9.5 mL, 54.70 mmol) in 55.0 mL of acetonitrile was added to a 250 mL one neck round bottom flask equipped with reflux condenser and drying tube. The mixture was refluxed for 24 h, then it was cooled to room temperature and concentrated under reduced pressure. The resultant oil was dissolved in methanol (55.0 mL) containing 2.95 g (54.7 mmol) of sodium methoxide. The mixture was stirred for 30 min and poured into saturated aqueous ammonium chloride (100.0 mL). The product was extracted with EtOAc (4×70 mL). The combined organic phase was dried over $Na_2SO_4$ and the solvent was removed under reduced pressure until dryness. The residue was dissolved in dichloromethane (11.0 mL) followed by sequential addition of imidazole (2.23 g, 32.76 mmol) and chlorotriethylsilane (2.75 mL, 16.47 mmol). The mixture was stirred for 6 h, then it was diluted with

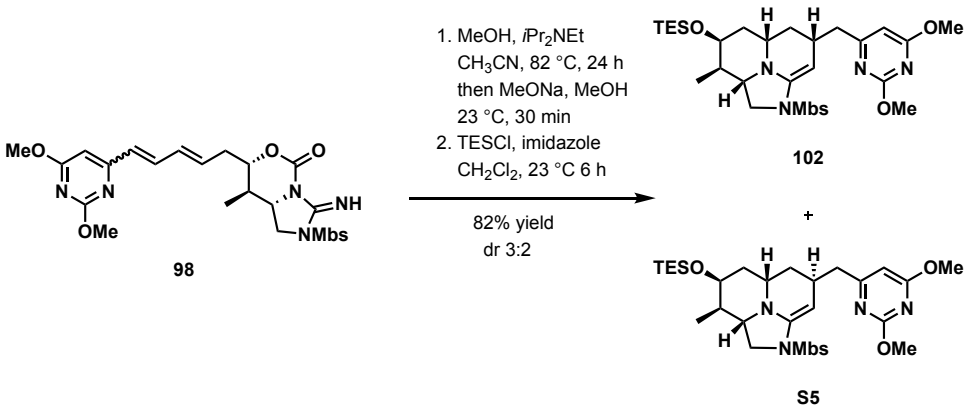
dichloromethane (50.0 mL) and poured in water (80.0 mL). The organic phase was separated and the aqueous phase was additionally extracted with dichloromethane (3×50mL). The combined organic solution was dried over $Na_2SO_4$ and dichloromethane was removed under reduced pressure. The desired isomer (1.55 g, 2.43 mmol, 44% yield) was isolated by column chromatography of crude product (65% EtOAc in hexanes → 50% EtOAc, 1% $Et_3N$ in acetone). Additional 1.68 g of cyclized product (93% combined yield) was isolated, that consisted of the mixture of stereoisomers in 85/15 ratio in favor of undesired one.

**Compound 102.**

$[\alpha]_{21}^{D}$- 39.5° (*c* 1.0, $CHCl_3$). $^1$H NMR (500 MHz, $CDCl_3$) δ 7.95 (d, *J* = 9.0 Hz, 2H), 6.89 (d, *J* = 9.0 Hz, 2H), 6.39 (s, 1H), 4.07 – 4.01 (m, 1H), 3.97 (s, 3H), 3.96 (s, 3H), 3.87 (s, 3H), 3.85 – 3.78 (m, 2H), 3.42 – 3.27 (m, 2H), 3.11 – 3.04 (m, 1H), 3.04 – 2.95 (m, 1H), 2.57 – 2.48 (m, 1H), 1.84 – 1.77 (m, 1H), 1.78 – 1.71 (m, 1H), 1.29 – 1.21 (m, 1H), 1.20 – 1.12 (m, 1H), 1.10 – 1.01 (m, 1H), 0.92 (t, *J* = 7.9 Hz, 9H), 0.89 (d, *J* = 6.8 Hz, 3H), 0.56 (q, *J* = 7.9 Hz, 6H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 171.86 (d, *J* = 8.8 Hz), 171.19, 165.14 (dd, *J* = 8.8, 6.7 Hz), 163.40, 147.38 (ddd, *J* = 16.9, 14.9, 9.6 Hz), 130.95, 129.52 (d, *J* = 3.8 Hz), 113.43, 101.81 – 100.05, 69.28, 55.61, 54.59, 53.76 (d, *J* = 3.4 Hz), 53.69, 52.98 (d, *J* = 7.6 Hz), 50.54 (d, *J* = 6.1 Hz), 46.23 (dd, *J* = 7.9, 5.5 Hz), 44.95 (d, *J* = 8.2 Hz), 40.94, 40.06, 34.44, 13.89, 6.95, 4.99. $^{15}$N NMR (41 MHz, $CDCl_3$) δ -148.18 (d, *J* = 1.3 Hz), -167.11 (d, *J* = 1.1 Hz), -206.15 (d, *J* = 4.6 Hz), -254.10 (dd, *J* = 4.8, 3.9 Hz), -288.49 (dd, *J* = 3.5, 0.9 Hz). HRMS-EI (m/z): $[M+H]^+$ calcd for $C_{30}H_{46}{}^{15}N_5O_6SSi$, 637.2790; found, 637.2792.

**Compound S5.**

$[\alpha]_{22}^{D}$ + 41.8° (*c* 1.0, $CHCl_3$). $^1$H NMR (500 MHz, $CDCl_3$) δ 7.91 (d, *J* = 8.9 Hz, 2H), 6.87 (d, *J* = 8.9 Hz, 2H), 6.25 (s, 1H), 3.96 – 3.89 (m, 7H), 3.85 (br. s, 1H), 3.82 (s, 3H), 3.80 – 3.75 (m, 1H), 3.52 – 3.44 (m, 1H), 3.37 (dd, *J* = 8.5, 6.3 Hz, 1H), 3.34 – 3.23 (m, 1H), 2.96 – 2.85 (m, 1H), 2.46 – 2.37 (m, 1H), 1.63 – 1.53 (m, 2H), 1.43 – 1.24 (m, 3H), 0.89 (t, *J* = 7.9 Hz, 9H), 0.85 (d, *J* = 6.7 Hz, 3H), 0.53 (q, *J* = 7.9 Hz, 3H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 171.85 (d, *J* = 8.8 Hz), 170.53, 165.10 (dd, *J* = 8.8, 6.5 Hz), 163.35, 147.06 (ddd, *J* = 17.5, 13.6, 9.7 Hz), 130.51, 129.84, 113.56, 100.76, 69.59, 55.58, 54.55 (dd, *J* = 3.1, 3.0 Hz), 53.96 (d, *J* = 6.8 Hz), 53.71 (d, *J* = 3.9 Hz), 51.12, 48.73 (d, *J* = 6.4 Hz), 45.46 (dd, *J* = 8.1, 2.8 Hz), 42.69 (d, *J* = 7.3 Hz), 39.21, 38.84, 30.51, 14.01 (d, *J* = 1.5 Hz), 6.92, 4.95. $^{15}$N NMR (41 MHz, cdcl$_3$) δ -148.62 (d, *J* = 1.1 Hz), -166.84 (d, *J* = 1.1 Hz), -203.68 (d, *J* = 5.3 Hz), -254.58 (dd, *J* = 5.1, 3.8 Hz), -289.37 (d, *J* = 3.5 Hz). HRMS-EI (m/z): $[M+H]^+$ calcd for $C_{30}H_{46}{}^{15}N_5O_6SSi$, 637.2790; found, 637.2783.




**The assembly of $^{14}N_5$-cylindrospermopsin core by means of double Michael addition.**

This synthetic sequence was performed using the procedure described above starting from 2.97 g (5.46 mmol) of compound **98**, 8.8 mL (0.218 mol) of MeOH, 9.5 ml (54.6 mmol) of

*i*$Pr_2$NEt and 45.5 ml of acetonitrile for the first step. Next methyl carbonate removal was affected using 1.26 g (54.6 mmol) of sodium in 54.5 ml of MeOH followed by silylation with 2.75 ml (16.4 mmol) chlorotriethylsilane and 2.23 g (32.8 mmol) of imidazole in 11.0 mL of dichloromethane. The desired isomer was isolated in 40% yield (1.36 g, 2.15 mmol). Additional 1.46 g of cyclized product (82% combined yield) was isolated, that consisted of the mixture of stereoisomers in 85/15 ratio in favor of undesired one.

**Compound 102.**

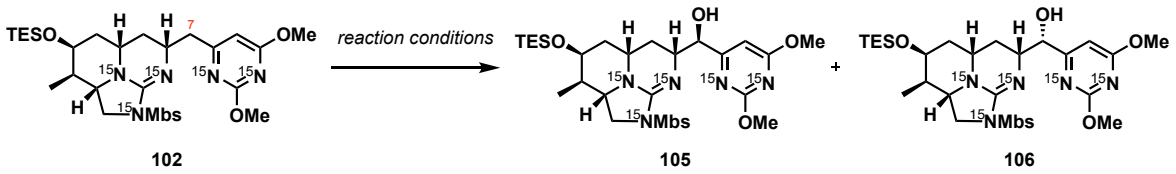
$[\alpha]_{20}^{D}$ - 38.7° (*c* 1.0, $CHCl_3$). $^1$H NMR (500 MHz, $CDCl_3$) δ 7.94 (d, *J* = 9.0 Hz, 2H), 6.89 (d, *J* = 9.0 Hz, 2H), 6.39 (s, 1H), 4.03 (dd, *J* = 8.6, 6.9 Hz, 1H), 3.96 (s, 3H), 3.96 (s, 3H), 3.86 (s, 3H), 3.85 – 3.78 (m, 2H), 3.42 – 3.27 (m, 2H), 3.07 (dd, *J* = 10.0, 8.8 Hz, 1H), 3.00 (dd, *J* = 13.3, 6.7 Hz, 1H), 2.52 (dd, *J* = 13.3, 7.4 Hz, 1H), 1.85 – 1.78 (m, 1H), 1.77 – 1.70 (m, 1H), 1.31 – 1.20 (m, 1H), 1.20 – 1.12 (m, 1H), 1.10 – 1.00 (m, 1H), 0.92 (t, *J* = 7.9 Hz, 9H), 0.89 (d, *J* = 6.8 Hz, 3H), 0.56 (q, *J* = 7.9 Hz, 6H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 171.80, 171.11, 165.10, 163.36, 147.37, 130.88, 129.39, 113.39, 100.80, 69.21, 55.55, 54.53, 53.70, 53.63, 52.94, 50.51, 46.15, 44.90, 40.86, 39.98, 34.38, 13.83, 6.89, 4.92. HRMS-EI (m/z): $[M+H]^+$ calcd for $C_{30}H_{46}N_5O_6SSi$, 632.2938; found, 632.2932.

**Compound S5.**

$[\alpha]_{21}^{D}$ + 37.2° (*c* 1.0, $CHCl_3$). $^1$H NMR (600 MHz, $CDCl_3$) δ 7.95 (d, *J* = 8.8 Hz, 2H), 6.90 (d, *J* = 8.8 Hz, 2H), 6.28 (s, 1H), 4.00 – 3.92 (m, 7H), 3.91 – 3.84 (m, 4H), 3.82 – 3.78 (m, 1H), 3.55 – 3.47 (m, 1H), 3.41 (dd, *J* = 9.2, 6.1 Hz, 1H), 3.36 – 3.26 (m, 1H), 2.93 (dd, *J* = 13.4, 6.0 Hz, 1H), 2.42 (dd, *J* = 13.4, 8.3 Hz, 1H), 1.65 – 1.58 (m, 2H), 1.45 – 1.39 (m, 1H), 1.38 – 1.28 (m, 2H), 0.93 (t, *J* = 7.9 Hz, 9H), 0.88 (d, *J* = 6.7 Hz, 3H), 0.56 (q, *J* = 8.0

Hz, 6H). $^{13}$C NMR (151 MHz, cdcl$_3$) δ 171.86, 170.53, 165.13, 163.36, 147.12, 130.52, 129.81, 113.57, 100.77, 69.59, 55.59, 54.56, 53.99, 53.72, 51.13, 48.76, 45.44, 42.71, 39.22, 38.85, 30.51, 14.01, 6.92, 4.96. HRMS-EI (m/z): $[M+H]^+$ calcd for $C_{30}H_{46}N_5O_6SSi$, 632.2938; found, 632.2924.

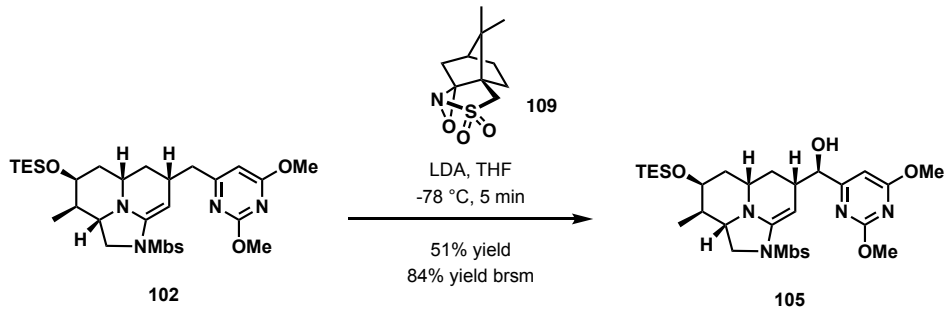
**Optimization of C-7 oxidation of compound 17 with Davis oxaziridines.**



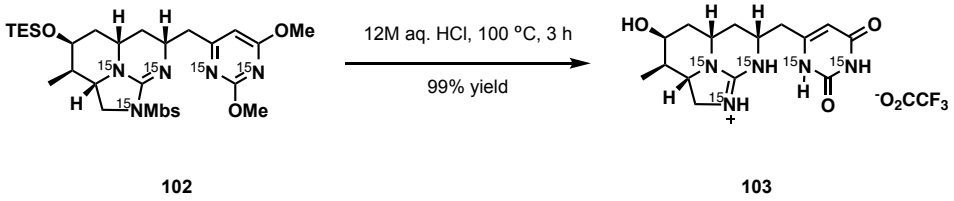

| Reaction conditions | Yield, % | 105/106 ratio |
|---|---|---|
| 2 eq. LDA, THF, -78 °C, 5 min<br>then 3 eq. 3-phenyl-2-(phenylsulfonyl)-1,2oxaziridine **107**, -78°C, 2 min | 22 | 6:1 |
| 2 eq. LDA, THF, -78 °C, 5 min<br>then 3 eq. (1*S*)-(+)-(camphorsulfonyl)oxaziridine **108**, -78°C, 2 min | 23 | 20:1 |
| 2 eq. LDA, -78 °C, 5 min<br>then 3 eq. (1*R*)-(-)-(camphorsulfonyl)oxaziridine **109**, -78°C, 2 min | 21 | 25:1 |
| 1.2 eq. LDA, -78 °C, 5 min<br>then 3 eq. (1*R*)-(-)-(camphorsulfonyl)oxaziridine **109**, -78°C, 2 min | 51<br>(84 brsm) | 25:1 |

**Compound-$^{15}N_5$, 105.** A solution of compound **102** (1.50 g, 2.36 mmol) in THF (47.0 mL) was added to a 250 mL one neck round bottom flask equipped with magnetic stirring bar and argon inlet adapter. In a separate flask, a solution of oxaziridine **109** (1.62 g, 7.06 mmol) in 10.0 mL of THF was prepared under argon. The reaction was cooled to -78 °C

and a freshly prepared LDA solution in THF (5.65 mL, 0.5 M) was added dropwise via syringe over 30 sec. During the addition, the solution changed from colorless to bright yellow. After stirring for an additional 2 min, the solution of oxaziridine **109** (1.62 g, 7.06 mmol) in THF was added via cannula into the reaction causing immediate color disappearance. The flask content was stirred for an additional 5 min and then quenched by addition of 1M solution of acetic acid in THF (5.0 mL). The product was extracted with EtOAc (3×70 mL), combined organic phase was dried over $Na_2SO_4$ and concentrated under vacuum. The desired product (0.71 g, 1.09 mmol, 46% yield, 78% brsm) and recovered starting material (612 mg, 0.96 mmol) were isolated by column chromatography (65% EtOAc in hexanes → 50% EtOAc, 1% $Et_3N$ in acetone). $[\alpha]_{21}^{D}$ + 46.1° (*c* 1.0, $CHCl_3$). $^1$H NMR (500 MHz, $CDCl_3$) δ 8.00 (d, *J* = 9.0 Hz, 2H), 6.99 (d, *J* = 9.0 Hz, 2H), 6.60 (s, 1H), 4.79 (s, 1H), 4.15 – 4.10 (m, 1H), 3.97 (s, 3H), 3.95 (s, 3H), 3.94 – 3.90 (m, 1H), 3.88 (s, 3H), 3.85 – 3.81 (m, 1H), 3.42 – 3.33 (m, 2H), 3.16 (dd, *J* = 10.2, 8.8 Hz, 1H), 3.02 (s, 1H), 1.77 – 1.68 (m, 1H), 1.45 – 1.37 (m, 1H), 1.33 – 1.25 (m, 1H), 1.19 – 1.06 (m, 2H), 0.96 – 0.90 (m, 12H), 0.60 – 0.53 (m, 6H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 174.52, 171.98 (d, *J* = 8.8 Hz), 164.92 (dd, *J* = 9.0, 6.7 Hz), 163.23, 147.59 (ddd, *J* = 17.9, 15.0, 10.1 Hz), 130.78, 130.58, 113.40, 99.62, 79.46 (dd, *J* = 9.3, 7.3 Hz), 69.39, 59.18, 55.54, 54.88 (dd, *J* = 3.1, 3.0 Hz ), 53.85 (d, *J* = 4.0 Hz), 53.14 (d, *J* = 7.8 Hz), 50.24 (d, *J* = 6.5 Hz), 44.92 (d, *J* = 8.2 Hz), 41.12, 40.49, 30.21, 13.90 (d, *J* = 2.0 Hz), 6.98, 6.89, 5.01, 4.87. $^{15}$N NMR (41 MHz, $CDCl_3$) δ -151.36 (d, *J* = 1.0 Hz), -164.38 (d, *J* = 1.1 Hz), -211.12 (d, *J* = 5.1 Hz), -255.52 (dd, *J* = 5.1, 4.1 Hz), -288.85 (d, *J* = 3.3 Hz). HRMS-EI (m/z): $[M+H]^+$ calcd for $C_{30}H_{46}{}^{15}N_5O_7SSi$, 653.2739; found, 653.2742.

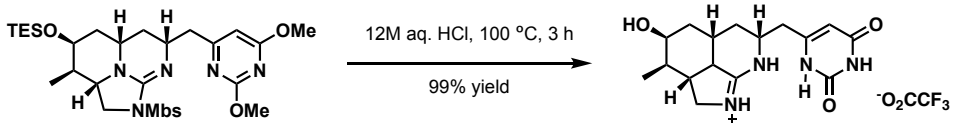



**Compound 105.** The title compound was obtained (0.60 g, 0.93 mmol, 51% yield) using the procedure described above for its $^{15}$N-analog starting from 1.15 g (1.82 mmol) of compound **102**, 4.4 ml (2.18 mmol) of 0.5M solution of LDA in THF, 1.25 g (5.45 mmol) of oxaziridine **109** and 55 ml THF. $[\alpha]_{21}^{D}$ + 44.1° (*c* 1.0, $CHCl_3$). $^1$H NMR (500 MHz, $CDCl_3$) δ 7.98 (d, *J* = 9.0 Hz, 2H), 6.97 (d, *J* = 9.0 Hz, 2H), 6.59 (s, 1H), 4.78 (d, *J* = 4.4 Hz, 1H), 4.11 (dd, *J* = 8.6, 6.9 Hz, 1H), 3.96 (s, 3H), 3.94 (s, 3H), 3.93 – 3.88 (m, 1H), 3.87 (s, 3H), 3.83 – 3.79 (m, 1H), 3.41 – 3.31 (m, 2H), 3.14 (dd, *J* = 10.3, 8.8 Hz, 1H), 3.03 (br. S, 1H), 1.74 – 1.69 (m, 1H), 1.43 – 1.36 (m, 1H), 1.31 – 1.23 (m, 1H), 1.18 – 1.04 (m, 2H), 0.95 – 0.88 (m, 12H), 0.54 (q, *J* = 7.9 Hz, 6H). $^{13}$C NMR (126 MHz, $CDCl_3$) δ 172.20, 171.61, 164.77, 163.58, 149.22, 130.46, 113.91, 98.40, 75.17, 69.07, 57.29, 55.64, 54.66, 53.86, 53.04, 50.65, 44.44, 40.91, 39.91, 29.68, 27.34, 13.84, 6.88, 4.90. HRMS-EI (m/z): $[M+H]^+$ calcd for $C_{21}H_{24}N_3O_4S$, 648.2887; found, 648.2908.




**Compound-$^{15}N_5$, 103.** The mixture of compound **102** (230 mg, 0.361 mmol) and 12M HCl (10.0 mL) was refluxed for 3 h. The reaction was cooled to room temperature and

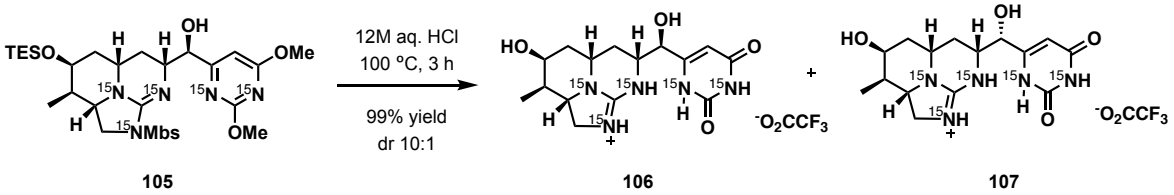
concentrated to dryness under vacuum. The crude product was purified by reversed-phase column chromatography on C18 reversed-phase silica gel (6% MeOH, 1% TFA in $H_2O$ → 25% MeOH, 1% TFA in $H_2O$) to produce the title compound (158 mg, 0.360 mmol, 99% yield) as amorphous white solid. $[\alpha]_{25}^{D}$ + 11.6° (*c* 1.0, MeOH). $^{1}$H NMR (600 MHz, $D_2O$) δ 5.67 (dd, *J* = 3.5, 2.8 Hz, 1H), 3.95 (br. s, 1H), 3.85 – 3.75 (m, 2H), 3.74 – 3.64 (m, 1H), 3.62 – 3.52 (m, 1H), 3.22 – 3.13 (m, 1H), 2.74 – 2.64 (m, 2H), 2.27 – 2.18 (m, 1H), 2.09 – 2.01 (m, 1H), 1.67 – 1.59 (m, 1H), 1.51 – 1.38 (m, 2H), 0.90 (d, *J* = 6.9 Hz, 3H). $^{13}$C NMR (126 MHz, $D_2O$) δ 166.65 (d, *J* = 10.8 Hz), 162.59 (q, *J* = 35.4 Hz), 154.78 (td, *J* = 21.9, 20.2 Hz), 153.18 (d, *J* = 13.3 Hz), 152.77 (dd, *J* = 19.8, 18.3 Hz), 116.30 (d, *J* = 291.8 Hz), 100.86 (d, *J* = 5.7 Hz), 67.82 (d, *J* = 1.3 Hz), 56.39 (d, *J* = 7.3 Hz), 47.51 (d, *J* = 9.1 Hz), 47.25 (d, *J* = 7.9 Hz), 43.93 (d, *J* = 8.1 Hz), 39.38, 37.29, 36.83, 33.06, 12.73 (d, *J* = 2.1 Hz). $^{15}$N NMR (41 MHz, $D_2O$) δ -223.97 (d, *J* = 2.4 Hz), -243.00, -280.91 (d, *J* = 2.7 Hz), -300.45, -306.60. HRMS-EI (m/z): $[M+H]^+$ calcd for $C_{15}H_{22}{}^{15}N_5O_3$, 325.1569; found, 325.1564.




**102** **103**

**Compound 103.** The title compound (37 mg, 0.085 mmol, 98% yield) was obtained from 50 mg (0.087 mmol) of compound **102** using the procedure described above for its $^{15}$N-analog. $[\alpha]_{23}^{D}$ + 12.3° (*c* 1.0, MeOH). $^{1}$H NMR (600 MHz, $D_2O$) δ 5.72 (s, 1H), 4.00 (s, 1H), 3.91 – 3.80 (m, 2H), 3.78 – 3.68 (m, 1H), 3.66 – 3.55 (m, 1H), 3.23 (dd, *J* = 10.8, 9.4 Hz, 1H), 2.74 (d, *J* = 6.9 Hz, 2H), 2.31 – 2.23 (m, 1H), 2.16 – 2.03 (m, 1H), 1.75 – 1.65

(m, 1H), 1.56 – 1.43 (m, 2H), 0.95 (d, $J$ = 6.9 Hz, 3H). $^{13}$C NMR (101 MHz, $D_2O$) δ 166.64, 162.60 (q, $J$ = 35.6 Hz), 154.70, 153.10, 152.72, 116.07 (q, $J$ = 291.4 Hz), 100.73, 67.71, 56.23, 47.37, 47.13, 43.78, 39.21, 37.11, 36.69, 32.89, 12.60. HRMS-EI (m/z): $[M+H]^+$ calcd for $C_{15}H_{22}N_5O_3$, 320.1717; found, 320.1727.




**Comprehensive hydrolysis of compound-$^{15}N_5$, 105.** The mixture of compound **105** (350 mg, 0.536 mmol) and 12M HCl (15.0 mL) was refluxed for 3 h. The reaction was cooled to room temperature and concentrated to dryness under vacuum. The crude product was purified by reversed-phase column chromatography on C18 reversed-phase silica gel (5% MeOH, 1% TFA in $H_2O$) to produce the mixture of C7-epimers **22** and **23** (242 mg, 0.533 mmol, dr 10:1, 99% yield) as amorphous white solid. A 30 mg portion of the mixture was separated by preparative HPLC to provide cylindrospermopsin-$^{15}N_5$ diol **106** and 7-*epi*-cylindrospermopsin-$^{15}N_5$ diol **107** for characterization purpose (column: Kinetex 5µm EVO C18 100Å column, 150×21.2 mm; eluent: 4% MeOH in $H_2O$ with 0.1% trifluoroacetic acid, flow rate: 10 mL/min, diode array detector: 262 nm).

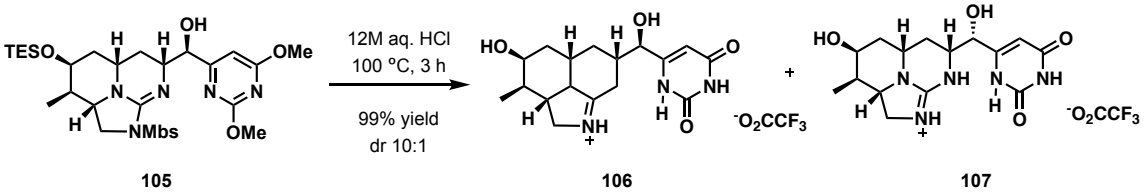
**Cylindrospermopsin-$^{15}N_5$ diol, 106.**

Amorphous white solid. $[\alpha]_{21}^{D}$ + 14.7° (*c* 1.0, MeOH). $^1$H NMR (600 MHz, $CD_3OD$) δ 5.65 (dd, $J$ = 3.5, 2.8 Hz, 1H), 4.51 (br. s, 1H), 3.84 (br. s, 1H), 3.81 - 3.68 (m, 3H), 3.66 – 3.57 (m, 1H), 3.21 – 3.12 (m, 1H), 2.13 – 1.98 (m, 2H), 1.64 – 1.48 (m, 2H), 1.46 – 1.38 (m, 1H), 0.94 (d, $J$ = 6.8 Hz, 3H). $^{13}$C NMR (151 MHz, $CD_3OD$) δ 166.81, 156.80 – 156.58

(m), 156.60 – 156.45 (m), 153.68 – 152.78 (m), 99.70, 70.72, 68.63 (d, $J$ = 4.4 Hz), 58.19 (d, $J$ = 7.0 Hz), 54.33 (d, $J$ = 9.3 Hz), 48.78, 45.47 (d, $J$ = 8.0 Hz), 41.51, 39.73, 29.53, 13.91. $^{15}$N NMR (41 MHz, $CD_3OD$) δ -224.43, -251.43, -280.93 (d, $J$ = 2.6 Hz), -303.81, -306.59. HRMS-EI (m/z): $[M+H]^+$ calcd for $C_{15}H_{22}{}^{15}N_5O_4$, 341.1518; found, 341.1534.

**7-*Epi*-cylindrospermopsin-$^{15}N_5$ diol, 107.**

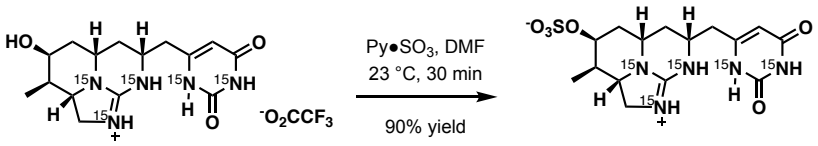
Amorphous white solid. $[\alpha]_{21}^{D}$ + 1.3° (*c* 0.5, MeOH). $^1$H NMR (600 MHz, $CD_3OD$) δ 5.72 (dd, $J$ = 3.5, 2.8 Hz, 1H), 4.30 (d, $J$ = 6.9 Hz, 1H), 3.92 (br. s, 1H), 3.89 – 3.76 (m, 2H), 3.77 – 3.62 (m, 2H), 3.28 – 3.18 (m, 1H), 2.18 – 2.04 (m, 2H), 1.68 – 1.56 (m, 2H), 1.51 – 1.44 (m, 1H), 1.02 (d, $J$ = 6.8 Hz, 3H). $^{13}$C NMR (101 MHz, $CD_3OD$) δ 166.83 (d, $J$ = 10.2 Hz), 156.59 (d, $J$ = 5.2 Hz), 156.50 (d, $J$ = 11.8 Hz), 153.57 – 153.10 (m), 99.96 (d, $J$ = 6.2 Hz), 72.60, 68.59, 58.17 (d, $J$ = 7.3 Hz), 54.02 (d, $J$ = 9.6 Hz), 48.87, 45.46 (d, $J$ = 8.1 Hz), 41.56, 39.71, 31.60, 13.92 (d, $J$ = 2.0 Hz). $^{15}$N NMR (41 MHz, $CD_3OD$) δ -224.26, -252.02, -281.07 (d, $J$ = 2.7 Hz), -304.76, -306.62. HRMS-EI (m/z): $[M+H]^+$ calcd for $C_{15}H_{22}{}^{15}N_5O_4$, 341.1518; found, 341.1534.




105 106 107

**Comprehensive hydrolysis of compound, 105.** The mixture of cylindrospermopsin diol **106** and 7-*epi*-cylindrospermopsin diol **107** (210 mg, 0.47 mmol, 99% yield) was obtained from 358 mg (0.47 mmol) of compound **105** using the procedure described above for its $^{15}$N-analog. The material was used for the next step without HPLC separation.

Cylindrospermopsin-$^{15}N_5$ diol, **106.** $^1$H NMR (500 MHz, $CD_3OD$) δ 5.71 (s, 1H), 4.56 (d, *J* = 4.3 Hz, 1H), 3.96 – 3.88 (m, 1H), 3.87 – 3.77 (m, 3H), 3.74 – 3.64 (m, 1H), 3.28 – 3.16 (m, 1H), 2.23 – 2.14 (m, 1H), 2.12 – 2.02 (m, 1H), 1.69 – 1.54 (m, 2H), 1.52 – 1.43 (m, 1H), 1.01 (d, *J* = 6.8 Hz, 3H). $^{13}$C NMR (151 MHz, $CD_3OD$) δ 166.78, 156.62, 156.48, 153.09, 99.67, 70.62, 68.56, 58.12, 54.25, 48.75, 45.39, 41.42, 39.65, 29.41, 13.84. HRMS-EI (m/z): $[M+H]^+$ calcd for $C_{15}H_{22}N_5O_4$, 336.1666; found, 336.1686.

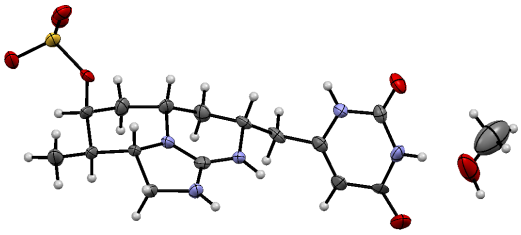


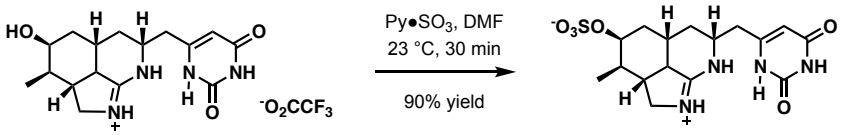

**103** **104**

**7-Deoxycylindrospermopsin-$^{15}N_5$, 104.** Compound **103** (27.0 mg, 0.062 mmol) was dissolved in DMF (1.2 mL) and added to a 5 mL one neck round bottom flask equipped with a magnetic stirring bar and argon inlet adapter. The solution was cooled to 0 °C under argon atmosphere and sulfur trioxide pyridine complex (49.0 mg, 0.308 mmol) was added in one portion. The reaction was stirred for 1 min at 0 °C, then allowed to warm to room temperature and stirred for another 30 min. After the quench with methanol (1.5 mL) the mixture was carefully concentrated by vacuum distillation and the residue was dissolved in 3 ml of methanol. The reaction mixture was left overnight without stirring. Formation of a white precipitate was observed and the solvent was carefully discarded using pipette. The precipitate was again mixed with 3 ml of methanol and after it was allowed to settle the solvent was again removed. The residue was dried overnight under vacuum to produce the desired product (22.1 mg, 90% yield) as white powder. $[\alpha]_{22}^{D}$ + 11.9° (*c* 0.4, DMSO). $^1$H NMR (600 MHz, DMSO-$D_6$) δ 10.99 (dd, *J* = 88.4, 1.5 Hz, 1H), 10.86 (dd, *J* = 73.7, 17.0

Hz, 1H), 8.37 (d, $J$ = 93.8 Hz, 1H), 7.71 (dd, $J$ = 96.0, 2.9 Hz, 1H), 5.45 (s, 1H), 4.28 (s, 1H), 3.90 – 3.73 (m, 2H), 3.63 – 3.54 (m, 2H), 3.13 – 3.08 (m, 1H), 2.61 – 2.53 (m, 2H), 2.48 – 2.40 (m, 1H), 2.24 – 2.11 (m, 1H), 1.72 – 1.62 (m, 1H), 1.35 – 1.20 (m, 2H), 0.90 (d, $J$ = 6.6 Hz, 3H). $^{13}$C NMR (151 MHz, DMSO-$D_6$) δ 164.01 (d, $J$ = 9.3 Hz), 154.40 (td, $J$ = 21.3, 19.8 Hz), 151.58 (dd, $J$ = 18.4, 16.9 Hz), 151.16 (d, $J$ = 10.9 Hz), 100.26 (d, $J$ = 6.0 Hz), 72.38 (d, $J$ = 6.5 Hz), 56.70 (d, $J$ = 7.2 Hz), 47.24 (d, $J$ = 9.0 Hz), 47.07 (d, $J$ = 7.4 Hz), 44.33 (d, $J$ = 7.5 Hz), 38.87, 36.35, 35.32, 32.89, 13.43. $^{15}$N NMR (41 MHz, DMSO-$D_6$) δ -223.19 (d, $J$ = 2.5 Hz), -244.17 (d, $J$ = 2.6 Hz), -280.94 (d, $J$ = 2.5 Hz), -297.35 (s), -304.30 (d, $J$ = 2.0 Hz). HRMS-EI (m/z): $[M+H]^+$ calcd for $C_{15}H_{22}N_5O_6S$, 405.1163; found, 405.1140.

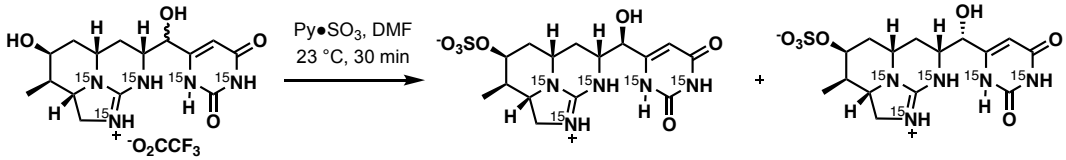
**Picture S3.** X-ray structure of 7-deoxycylindrospermopsin **104** (cocrystal with methanol).






103 104

**7-Deoxycylindrospermopsin, 104.** The title compound (19.5 mg, 0.049 mmol, 88% yield) was obtained from 24 mg (0.055 mmol) of compound **103** and sulfur trioxide pyridine complex (44.0 mg, 0.277 mmol) in 1.1 ml of DMF using the procedure described above for its $^{15}$N-analog. White powder. $[\alpha]_{21}^{D}$ + 10.2° (*c* 0.4, DMSO). $^{1}$H NMR (500 MHz, DMSO-$D_6$) δ 10.99 (s, 1H), 10.85 (s, 1H), 8.37 (s, 1H), 7.72 (s, 1H), 5.45 (s, 1H), 4.28 (s, 1H), 3.88 – 3.72 (m, 2H), 3.65 – 3.54 (m, 2H), 3.15 – 3.05 (m, 1H), 2.55 (d, *J* = 6.5 Hz, 2H), 2.49 – 2.39 (m, 1H), 2.23 – 2.11 (m, 1H), 1.76 – 1.58 (m, 1H), 1.38 – 1.18 (m, 2H), 0.90 (d, *J* = 6.7 Hz, 3H). $^{13}$C NMR (126 MHz, DMSO-$D_6$) δ 164.09, 154.48, 151.64, 151.25, 100.31, 72.50, 56.76, 47.31, 47.13, 44.39, 38.91, 36.40, 35.36, 32.94, 13.46. HRMS-EI (m/z): $[M+H]^+$ calcd for $C_{15}H_{22}N_5O_6S$, 400.1291; found, 400.1303.




**105+106** (10:1) **4**, 77% yield **108**, 7% yield

**Chemoselective sulfation reaction.** A mixture of diols **105** and **106** (105 mg, 0.231 mmol, dr 10:1) was dissolved in DMF (4.6 mL) and added to a 25 mL one neck round bottom flask equipped with stirring bar and argon inlet adapter. The solution was cooled to 0 °C under an atmosphere of argon and sulfur trioxide pyridine complex (111 mg, 0.697 mmol) was added in one portion. The reaction was stirred for 1 min at 0 °C, then allowed to warm to room temperature and stirred for another 30 min. After the quench with methanol (3.5

mL) the mixture was carefully concentrated by vacuum distillation and the crude product was fractionated using preparative HPLC to deliver cylindrospermopsin-$^{15}N_5$ **4** (75.0 mg, 77% yield) and 7-*epi*-cylindrospermopsin-$^{15}N_5$ **108** (6.8 mg, 7% yield) (column: Kinetex 5µm EVO C18 100Å column, 150×21.2 mm; eluent: $H_2O$ with 0.1% trifluoroacetic acid, flow rate: 10 mL/min, diode array detector: 262 nm).

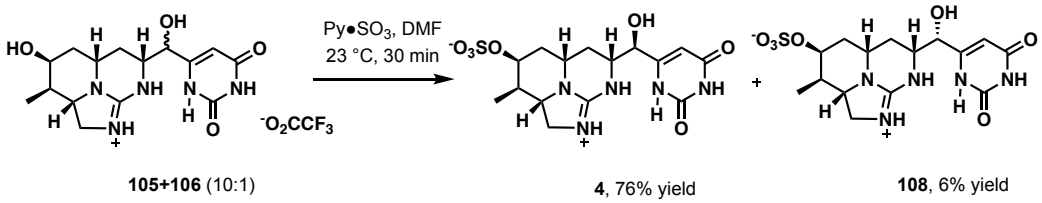
**Cylindrospermopsin-$^{15}N_5$, 4**.

White powder. $[\alpha]_{23}^{D}$ + 13.5° (*c* 1.0, $H_2O$). $^1$H NMR (600 MHz, $D_2O$) δ 5.87 (dd, *J* = 3.3, 2.8 Hz, 1H), 4.78 (m, 1H), 4.64 (br. s, 1H), 3.96 – 3.83 (m, 2H), 3.85 – 3.74 (m, 1H), 3.73 – 3.64 (m, 1H), 3.33 – 3.24 (m, 1H), 2.53 – 2.44 (m, 1H), 2.28 – 2.14 (m, 1H), 1.99 – 1.86 (m, 1H), 1.68 – 1.51 (m, 2H), 1.02 (d, *J* = 6.8 Hz, 3H). $^{13}$C NMR (126 MHz, $D_2O$) δ 166.57 (d, *J* = 10.9 Hz), 155.27 (d, *J* = 20.8 Hz), 154.93 (d, *J* = 12.1 Hz), 152.70 – 152.31 (m), 98.83 (d, *J* = 5.4 Hz), 76.96, 69.05, 56.66 (d, *J* = 7.3 Hz), 52.38 (d, *J* = 9.6 Hz), 47.11 (d, *J* = 8.5 Hz), 43.78 (d, *J* = 7.7 Hz), 38.56, 35.06, 27.18, 12.50. $^{15}$N NMR (41 MHz, DMSO-$D_6$) δ -222.54 (d, *J* = 2.5 Hz), -249.06 (d, *J* = 2.6 Hz), -281.34 (d, *J* = 2.5 Hz), -300.80 (s), -304.27 (d, *J* = 2.0 Hz). HRMS-EI (m/z): $[M+H]^+$ calcd for $C_{15}H_{22}{}^{15}N_5O_7S$, 421.1092; found, 421.1147.

**7-*Epi*-cylindrospermopsin-$^{15}N_5$, 108.**

White powder. $[\alpha]_{22}^{D}$ + 1.0° (*c* 0.5, $H_2O$). $^1$H NMR (600 MHz, $D_2O$) δ 5.87 (dd, *J* = 3.3, 2.8 Hz, 1H), 4.65 (br. s, 1H), 4.53 (d, *J* = 6.3 Hz, 1H), 3.91 – 3.86 (m, 1H), 3.85 – 3.75 (m, 2H), 3.75 – 3.68 (m, 1H), 3.34 – 3.25 (m, 1H), 2.57 – 2.45 (m, 1H), 2.30 – 2.15 (m, 1H), 1.96 – 1.86 (m, 1H), 1.74 – 1.63 (m, 1H), 1.61 – 1.53 (m, 1H), 1.03 (d, *J* = 6.8 Hz, 3H). $^{13}$C NMR (126 MHz, $D_2O$) δ 166.65 (d, *J* = 10.9 Hz), 155.23 (d, *J* = 17.5 Hz), 155.11 (d, *J*

= 12.0 Hz), 152.99 – 152.47 (m), 99.18 (d, *J* = 5.7 Hz), 77.01, 70.61, 56.67 (d, *J* = 7.3 Hz), 52.13 (d, *J* = 9.5 Hz), 47.20 (d, *J* = 8.1 Hz), 43.80 (d, *J* = 8.2 Hz), 38.63, 35.08, 29.50, 12.57 (d, *J* = 1.9 Hz). $^{15}$N NMR (41 MHz, DMSO-$D_6$) δ -222.48 (d, *J* = 2.5 Hz), -249.93 (d, *J* = 2.3 Hz), -281.31 (d, *J* = 2.4 Hz), -302.04 (s), -303.75 (d, *J* = 2.4 Hz). HRMS-EI (m/z): $[M+H]^+$ calcd for $C_{15}H_{22}{}^{15}N_5O_7S$, 421.1092; found, 421.1147.




**Chemoselective sulfation reaction.** This reaction was performed starting from the mixture of C-7 epimers **105** and **106** (135 mg, 0.30 mmol) and sulfur trioxide pyridine complex (144.0 mg, 0.90 mmol) in 6 ml of DMF using the procedure described above for its $^{15}$N-analog. After the purification cylindrospermopsin **4** (94.8 mg, 0.228 mmol, 76% yield) and 7-*epi*-cylindrospermopsin **108** (7.5 mg, 0.018 mmol, 6% yield) were obtained as white solids.

**Cylindrospermopsin, 4**.

White powder. $[\alpha]_{24}^{D}$ + 14.7° (*c* 1.0, $H_2O$). $^1$H NMR (600 MHz, $D_2O$) δ 5.86 (s, 1H), 4.77 (d, *J* = 4.1 Hz, 1H), 4.64 (s, 1H), 3.97 – 3.85 (m, 2H), 3.82 – 3.74 (m, 1H), 3.72 – 3.60 (m, 1H), 3.31 – 3.22 (m, 1H), 2.54 – 2.36 (m, 1H), 2.26 – 2.13 (m, 1H), 1.96 – 1.84 (m, 1H), 1.66 – 1.49 (m, 2H), 1.02 (d, *J* = 6.8 Hz, 3H). $^{13}$C NMR (126 MHz, $D_2O$) δ 166.58, 155.34, 155.00, 152.52, 98.90, 76.97, 69.12, 56.76, 52.50, 47.21, 43.89, 38.64, 35.15, 27.27, 12.60. HRMS-EI (m/z): $[M+H]^+$ calcd for $C_{15}H_{22}N_5O_7S$, 416.1240; found, 416.1292.

**7-*Epi*-cylindrospermopsin, 108.**

White powder. $[\alpha]_{22}^{D}$ + 1.0° (*c* 0.5, $H_2O$). $^1$H NMR (600 MHz, $D_2O$) δ 5.87 (s, 1H), 4.65 (s, 1H), 4.53 (d, *J* = 6.3 Hz, 1H), 3.96 – 3.85 (m, 1H), 3.85 – 3.75 (m, 2H), 3.75 – 3.69 (m,

1H), 3.34 – 3.23 (m, 1H), 2.57 – 2.44 (m, 1H), 2.31 – 2.16 (m, 1H), 1.99 – 1.85 (m, 1H), 1.73 – 1.62 (m, 1H), 1.62 – 1.52 (m, 1H), 1.03 (d, $J$ = 6.8 Hz, 3H). $^{13}$C NMR (126 MHz, $D_2O$) δ 166.66, 155.26, 155.13, 152.72, 99.19, 77.01, 70.61, 56.69, 52.16, 47.23, 43.83, 38.64, 35.09, 29.51, 12.58. HRMS-EI (m/z): $[M+H]^+$ calcd for $C_{15}H_{22}N_5O_7S$, 416.1240; found, 416.1250.

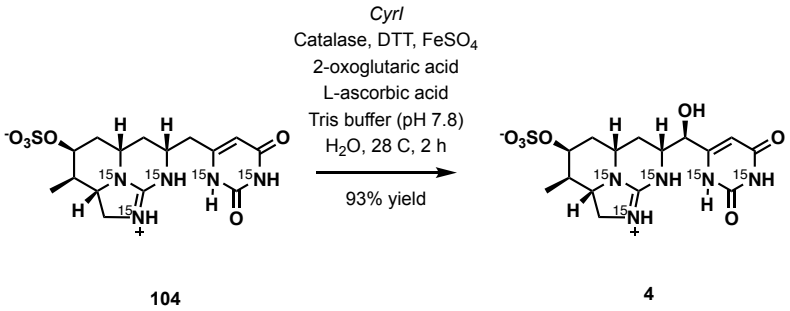
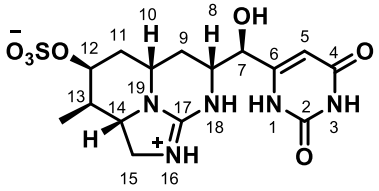



**Table S1.** Comparison of $^1$H NMR Data of Synthetic and Reported Cylindrospermopsin ($D_2O$)



| Position | Synthetic 1 (600 MHz) | Reported for natural 1 (500 MHz) |
|---|---|---|
| 5 | 5.86 (s, 1H) | 5.83 (s, 1H) |
| 7 | 4.77 (d, $J$ = 4.1 Hz, 1H) | 4.70 (d, $J$ = 3.9 Hz, 1H) |
| 12 | 4.64 (br. s, 1H) | 4.60 (br. s, 1H) |
| 8 | 3.90 (ddd, $J$ = 11.7, 3.7, 3.4 Hz, 1H) | 3.87 (ddd, $J$ = 11.4, 3.9, 3.6 Hz, 1H) |
| 15β | 3.88 (dd, $J$ = 9.0, 9.5 Hz, 1H) | 3.84 (dd, $J$ = 9.2, 8.8 Hz, 1H) |

| | | |
|---|---|---|
| 14 | 3.77 (ddd, *J* = 11.0, 10.8, 9.0 Hz, 1H) | 3.74 (ddd, *J* = 11.1, 11.1, 8.8 Hz, 1H) |
| 10 | 3.69 (ddt, *J* = 11.4, 11.2, 3.6 Hz, 1H) | 3.66 (ddt, *J* = 11.8, 11.4, 3.6 Hz, 1H) |
| 15α | 3.28 (dd, *J* = 10.8, 9.5 Hz, 1H) | 3.25 (dd, *J* = 11.1, 9.2 Hz, 1H) |
| 11β | 2.48 (ddd, *J* = 14.2, 3.6, 1.9 Hz, 1H) | 2.45 (ddd, *J* = 14.4, 3.6, 2.0 Hz, 1H) |
| 9 β | 2.19 (dt, *J* = 13.3, 3.5 Hz, 1H) | 2.16 (dt, *J* = 13.4, 3.6 Hz, 1H) |
| 12 | 1.88 (dqd, *J* =10.6, 7.6, 1.8 Hz, 1H) | 1.85 (dqd, *J* = 11.1, 6.9, 2.3 Hz, 1H) |
| 9α | 1.60 (ddd, *J* = 12.8, 11.9, 11.9 Hz, 1H) | 1.57 (ddd, *J* = 13.4, 11.4, 11.4 Hz, 1H) |
| 11α | 1.58 (ddd, *J* = 14.3 Hz, 11.5, 3.5 Hz, 1H) | 1.52 (ddd, *J* = 14.4, 11.8, 3.6 Hz, 1H) |
| $CH_3$ | 1.02 (d, *J* = 6.8, 3H) | 0.99 (d, *J* = 6.8, 3H) |

**Enzymatic oxidation of 7-deoxycylindrospermopsin 3 to cylindrospermopsin 1.**

*Optimization of enzymatic oxidation conditions.*

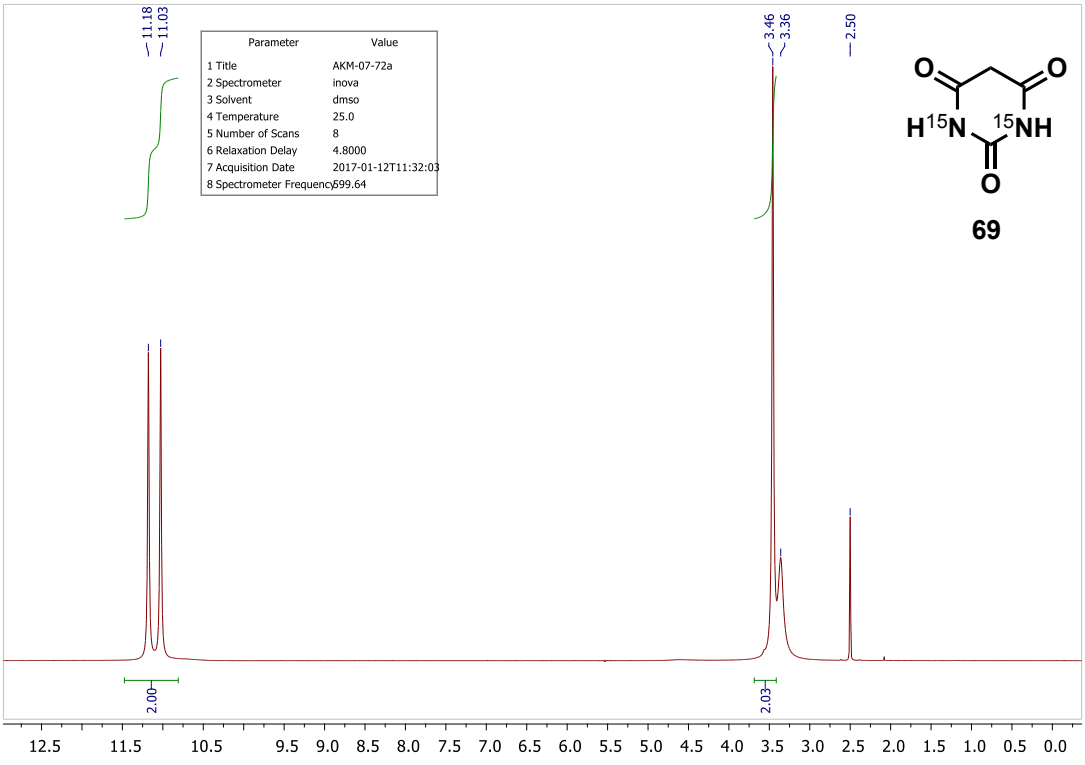
Table **S2**. Optimization of the enzymatic oxidation of deoxycylindrospermopsin.

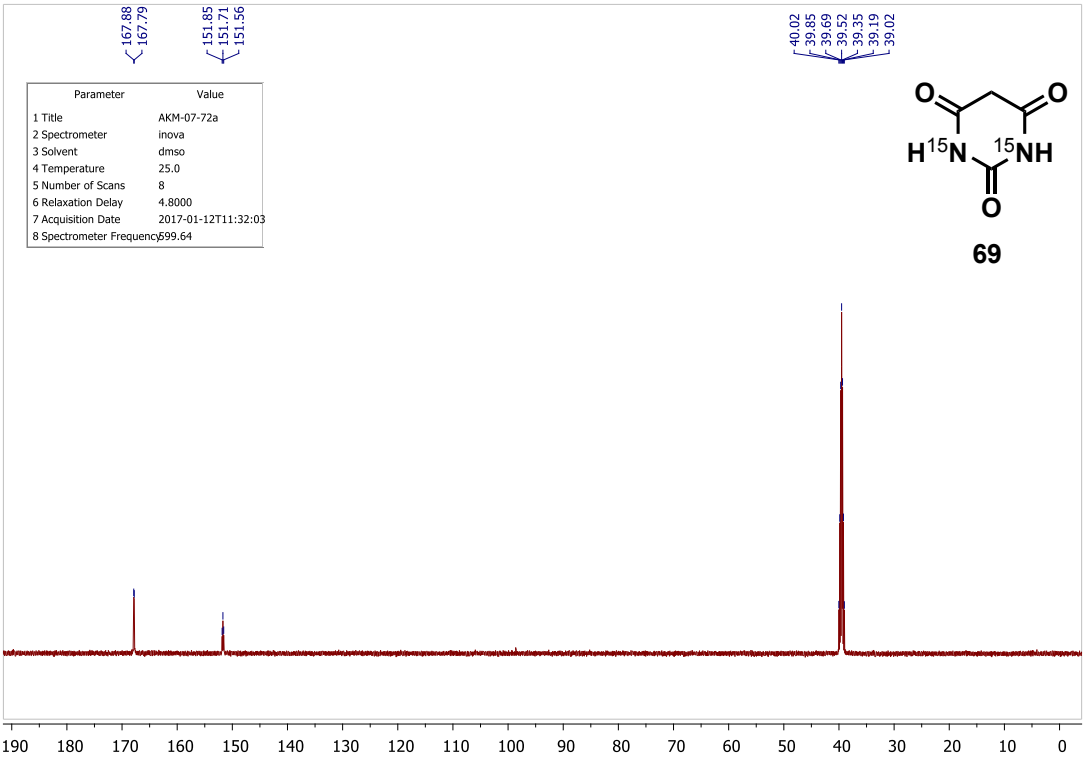
| Entry | Substrate concentration in the reaction mixture, (μM/L) | Biocatalyst loading, (mol%) | Conversion, (%) |
|---|---|---|---|
| 1 | 20 | 25 | 100 |
| 2 | 40 | 12.5 | 100 |
| 3 | 100 | 5 | 100 |
| 4 | 200 | 2.5 | 95 |
| 5 | 300 | 1.7 | 40 |
| 6 | 400 | 1.3 | 22 |
| 7 | 1000 | 0.5 | 7 |

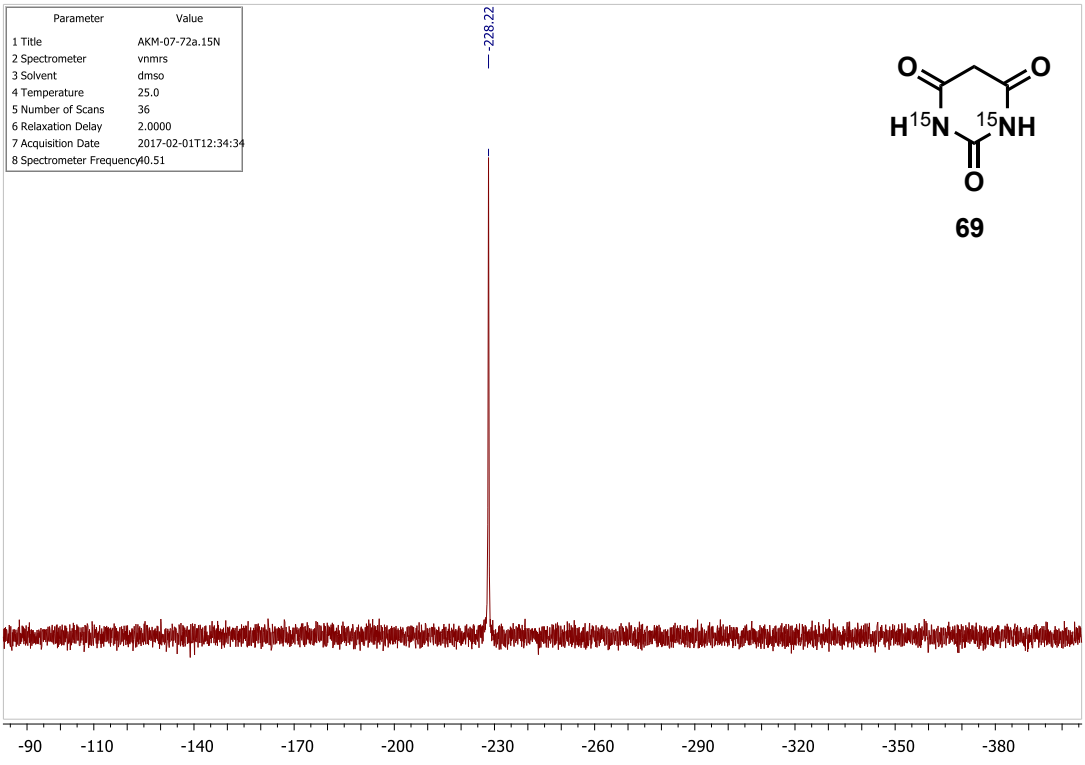
Due to the low solubility, the stock solution of 7-deoxycylindrospermopsin (10mM/L) was prepared by dissolving 4 mg of the compound in 0.1 mL of DMSO at 50°C followed by dilution with water (HPLC grade) to 1 mL volume. Working solutions (200, 400, 1000, 2000, 3000, 4000 μM/L) were prepared by diluting the standard solution with HPLC grade water.

The procedure reported by Ploux *et al.* was adopted with some modifications.[1] In 1.5 mL microcentrifuge tube 100 μL of water (HPLC grade) was added followed by 50 μL of solution of each following component in water: catalase (1000 μg/mL), 1,4-dithiothreitol (DTT, 1000 μM/L), $FeSO_4$ (500 μM/L), 2-oxoglutaric acid (5 mM/L), L-ascorbic acid (20 mM/L) and TrisHCl buffer (pH 7.8, 500 mM/L). Next a corresponding working solution of substrate (50 μL) was added followed by *CyrI* protein (50 μL, 50μM/L). The reaction thoroughly stirred on vortex mixer and incubated at 28 °C for 2 h. Next the content was heated at 100 °C for 5 min inducing proteins denaturation. After centrifugation (10000 rpm, Thermo Scientific Legend Micro 21R) the sample was analyzed using HPLC coupled with UV-Vis detector (column: Kinetex 5μm EVO C18 100Å column, 150×21.2 mm; eluent: 1% $CH_3CN$ in $H_2O$ with 0.1% trifluoroacetic acid, flow rate: 1.3 mL/min, diode array detector: 262 nm, 7-*epi*-cylindrospermopsin - 2.96 min, cylindrospermopsin - 3.41 min, 7-deoxycylindrospermopsin – 12.31 min).

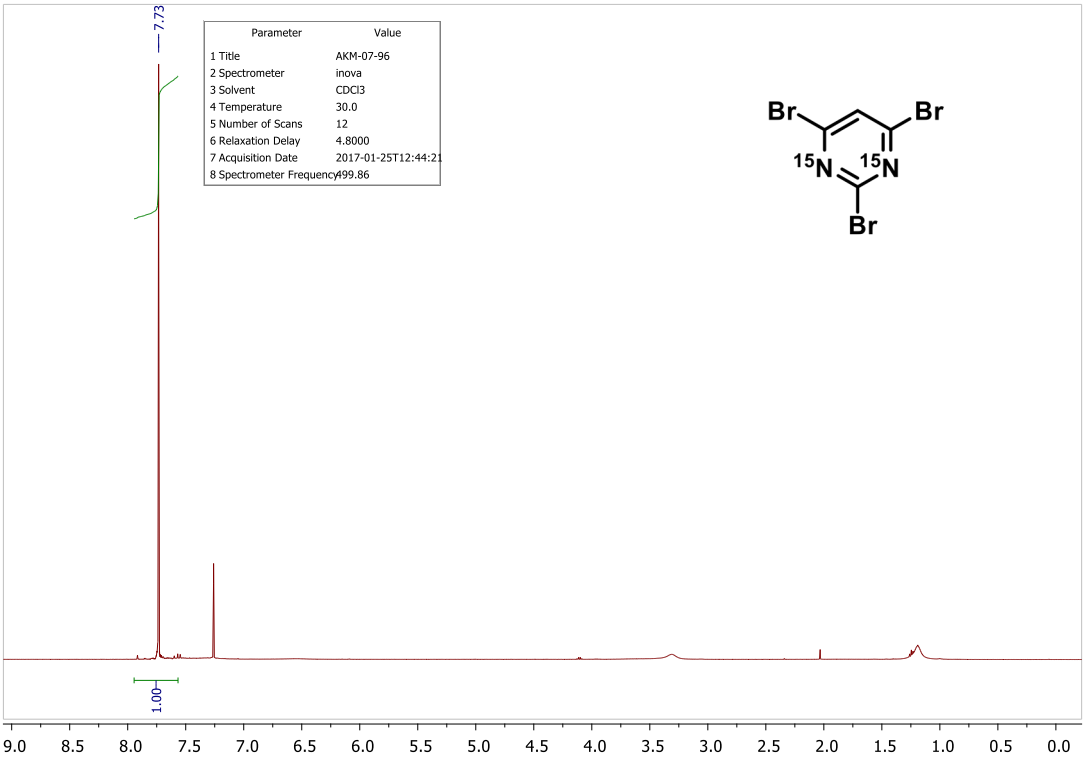
[1] Mazmouz, R.; Chapuis-Hugon, F.; Pichon, V.; Mejean, A.; Ploux, O. *ChemBioChem*, **2011**, *12*, 858.

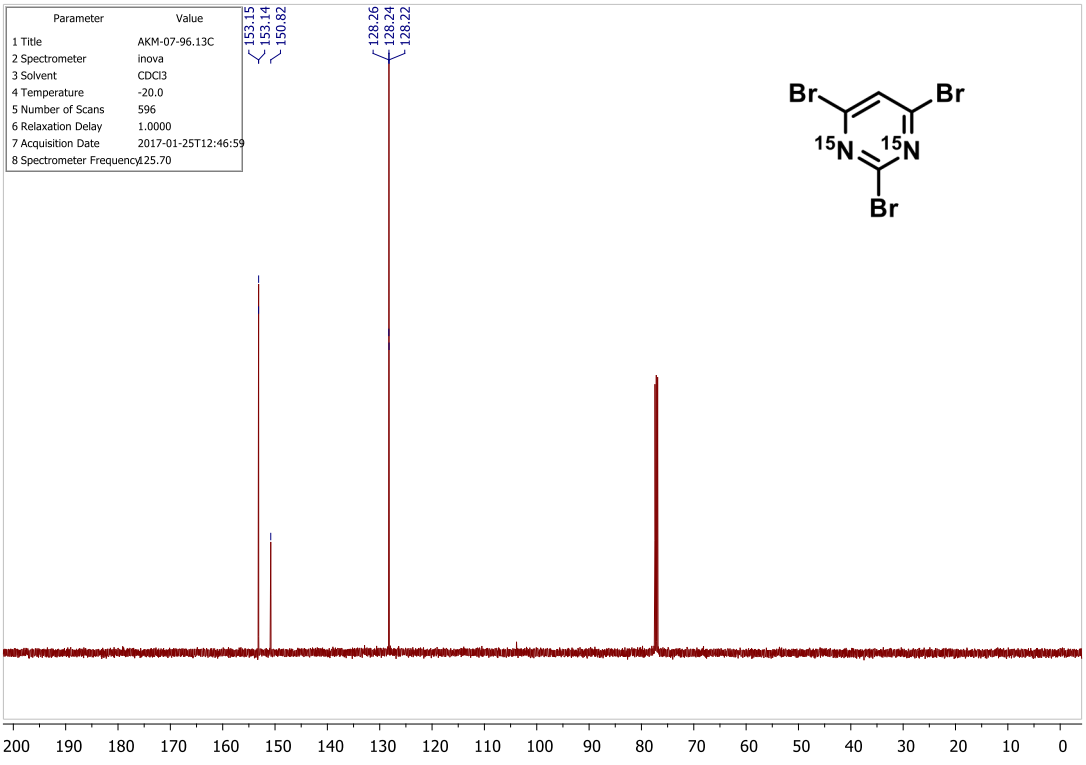

11.18
11.03
3.46
3.36
2.50
Parameter Value
1 Title AKM-07-72a
2 Spectrometer inova
3 Solvent dmso
4 Temperature 25.0
5 Number of Scans 8
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-01-12T11:32:03
8 Spectrometer Frequency 599.64
O
O
H15N
15NH
O
69
2.00
2.03
12.5 11.5 10.5 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

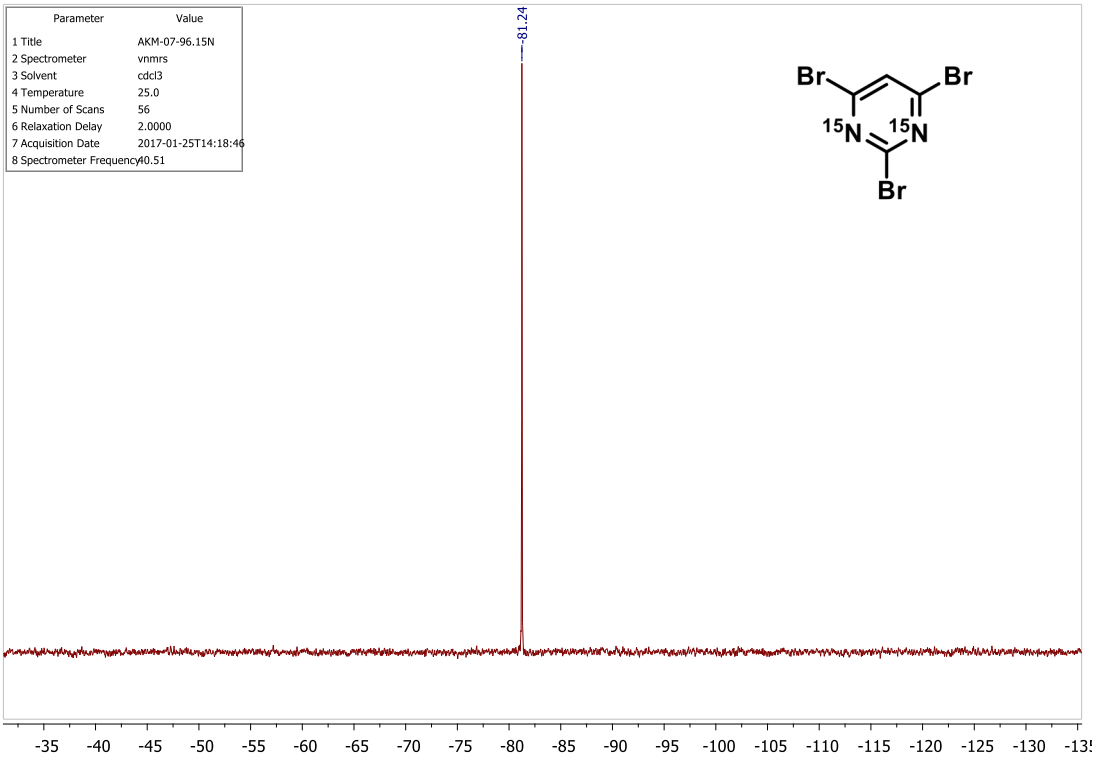

167.88
167.79
151.85
151.71
151.56
40.02
39.85
39.69
39.52
39.35
39.19
39.02
Parameter Value
1 Title AKM-07-72a
2 Spectrometer inova
3 Solvent dmso
4 Temperature 25.0
5 Number of Scans 8
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-01-12T11:32:03
8 Spectrometer Frequency 599.64
O
O
H15N
15NH
O
69
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

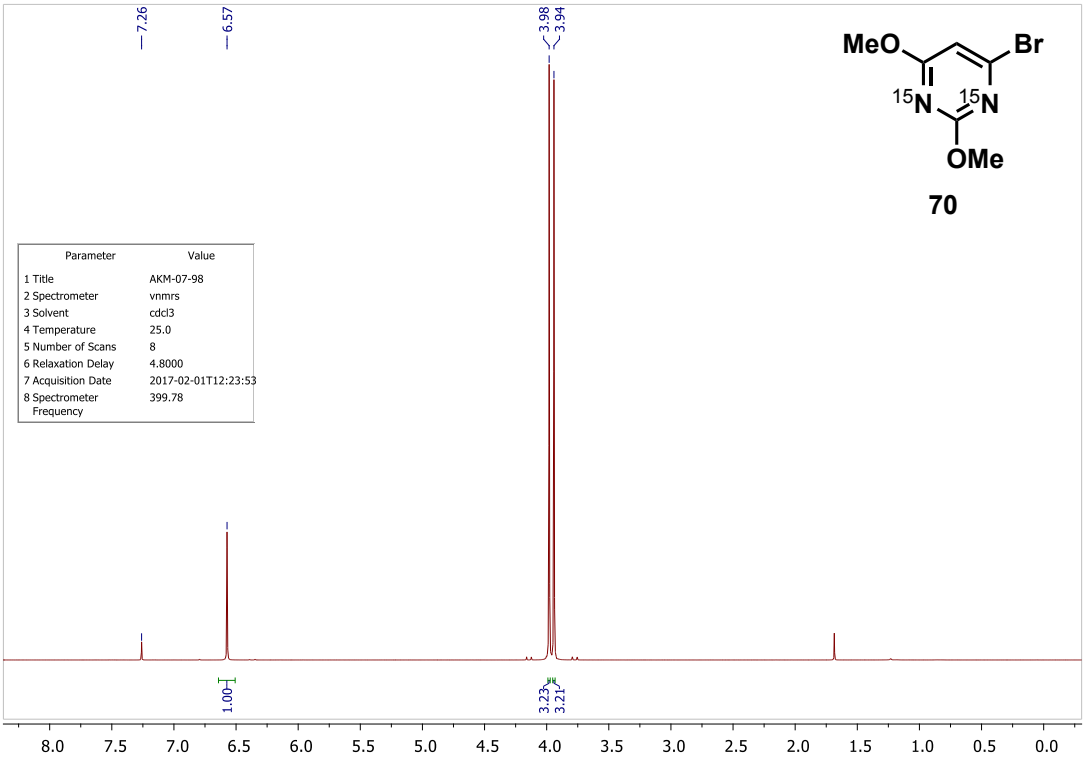

Parameter Value
1 Title AKM-07-72a.15N
2 Spectrometer vnmrs
3 Solvent dmso
4 Temperature 25.0
5 Number of Scans 36
6 Relaxation Delay 2.0000
7 Acquisition Date 2017-02-01T12:34:34
8 Spectrometer Frequency 40.51
-228.22
O
O
H15N
15NH
O
-90 -110 -140 -170 -200 -230 -260 -290 -320 -350 -380

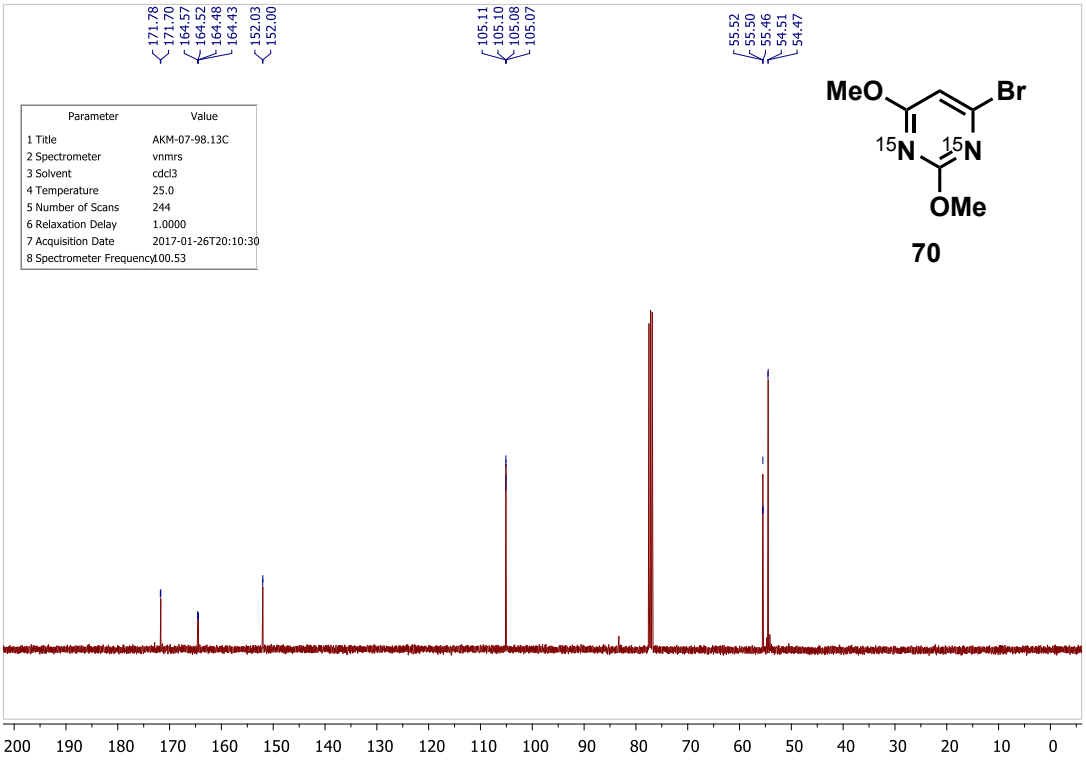
**69**

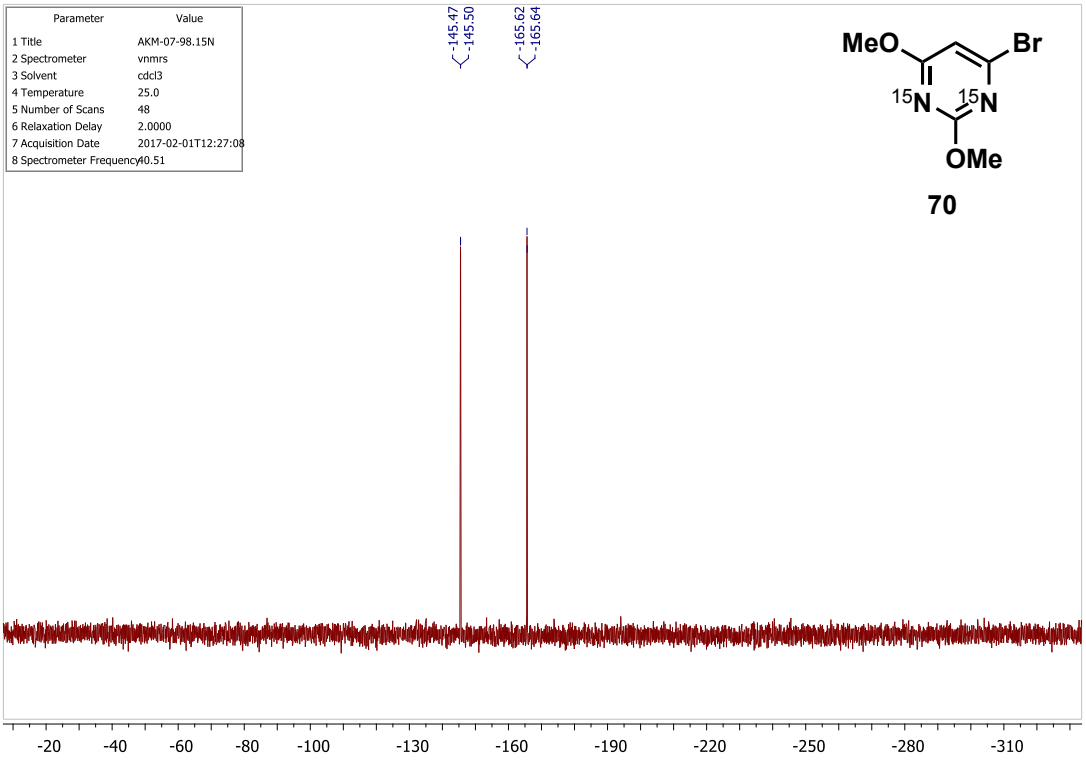

7.73
Parameter Value
1 Title AKM-07-96
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 30.0
5 Number of Scans 12
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-01-25T12:44:21
8 Spectrometer Frequency 499.86
Br
Br
15N
15N
Br
1.00
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

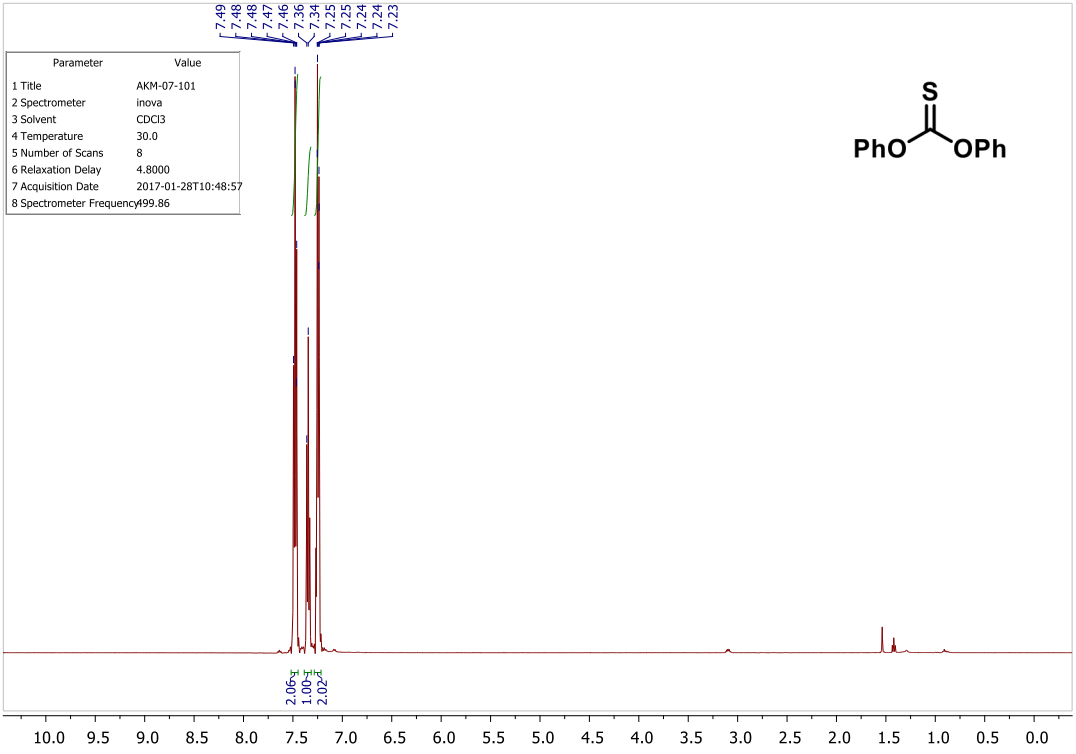

Parameter Value
1 Title AKM-07-96.13C
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature -20.0
5 Number of Scans 596
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-01-25T12:46:59
8 Spectrometer Frequency 125.70
153.15
153.14
150.82
128.26
128.24
128.22
Br
Br
15N
15N
Br
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

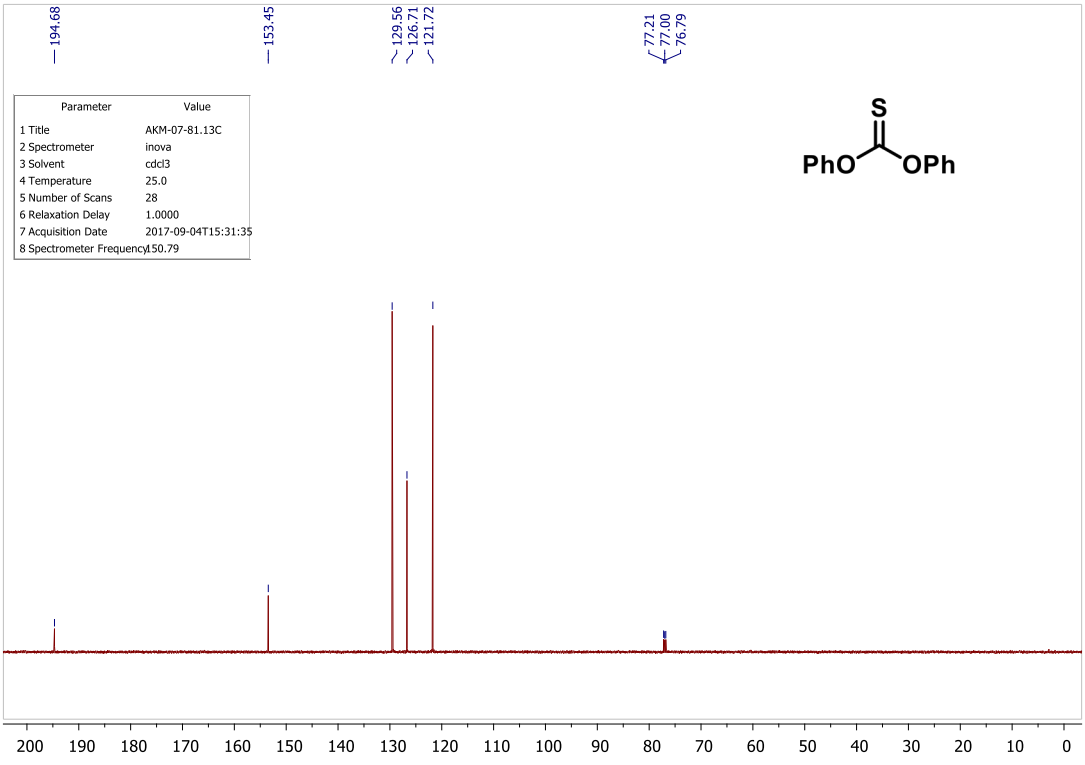

Parameter Value
1 Title AKM-07-96.15N
2 Spectrometer vnmrs
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 56
6 Relaxation Delay 2.0000
7 Acquisition Date 2017-01-25T14:18:46
8 Spectrometer Frequency 40.51
81.24
Br
Br
15N
15N
Br
-35 -40 -45 -50 -55 -60 -65 -70 -75 -80 -85 -90 -95 -100 -105 -110 -115 -120 -125 -130 -13

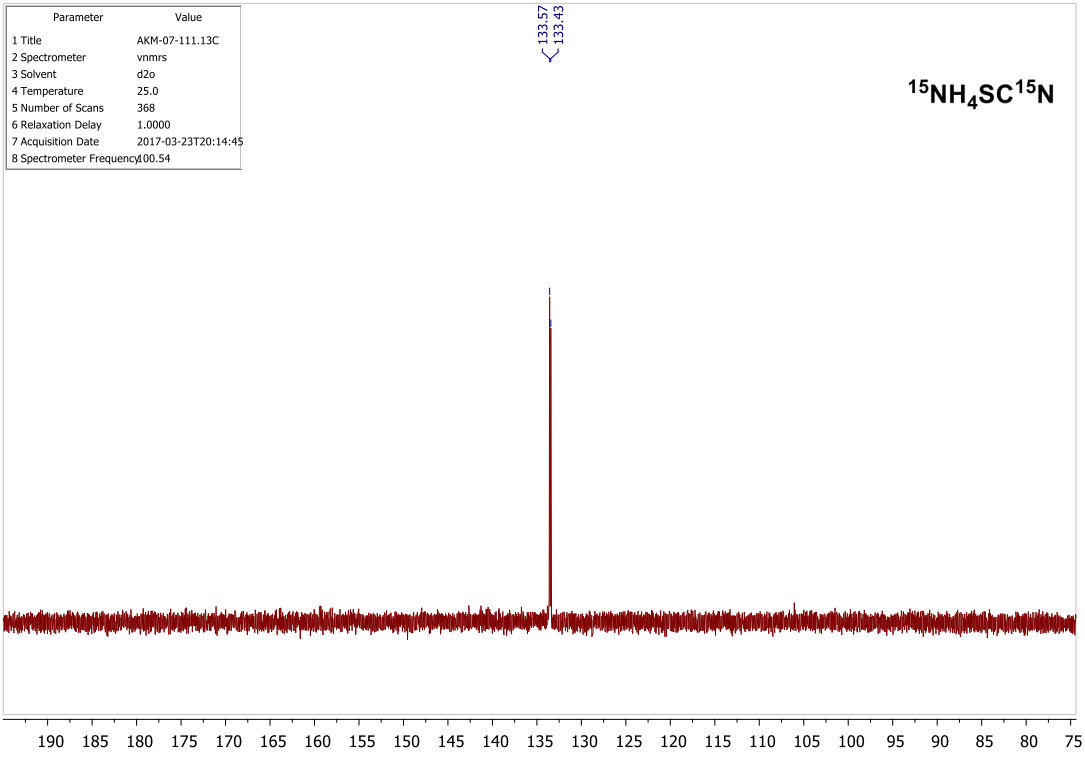

7.26
6.57
3.98
3.94
MeO
Br
15N
15N
OMe
70
Parameter Value
1 Title AKM-07-98
2 Spectrometer vnmrs
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 8
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-02-01T12:23:53
8 Spectrometer Frequency 399.78
1.00
3.23
3.21
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

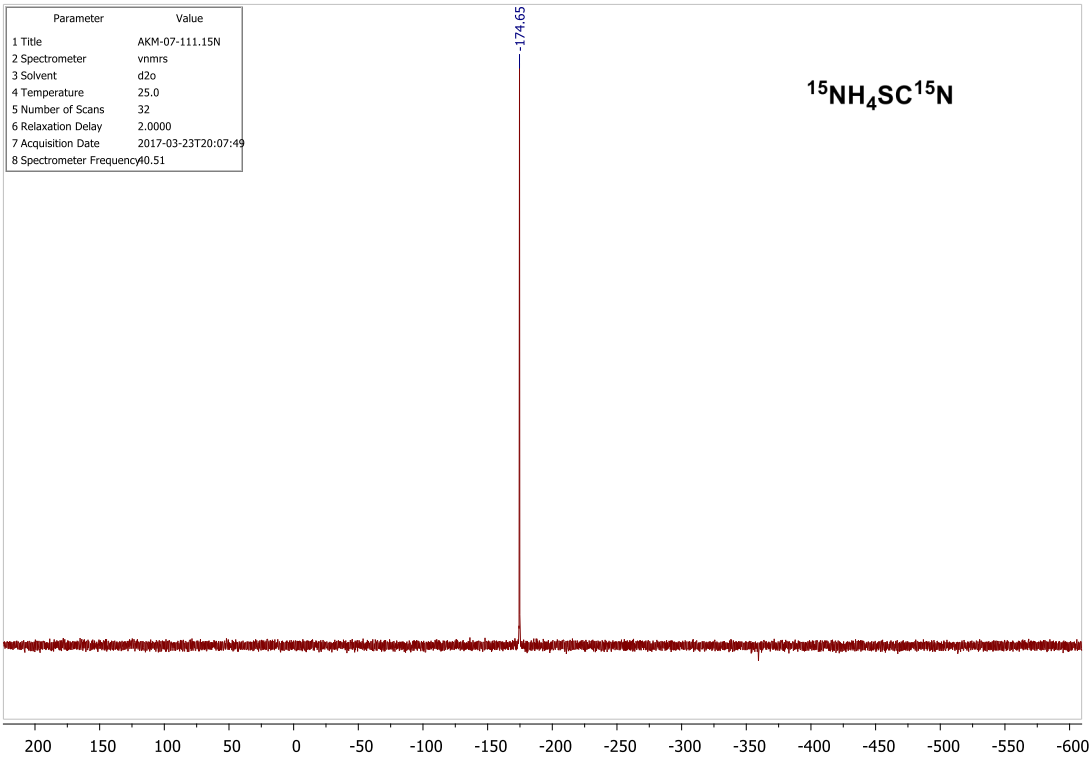

171.78
171.70
164.57
164.52
164.48
164.43
152.03
152.00
105.11
105.10
105.08
105.07
55.52
55.50
55.46
54.51
54.47
MeO
Br
15N
15N
OMe
70
Parameter Value
1 Title AKM-07-98.13C
2 Spectrometer vnmrs
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 244
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-01-26T20:10:30
8 Spectrometer Frequency 100.53
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

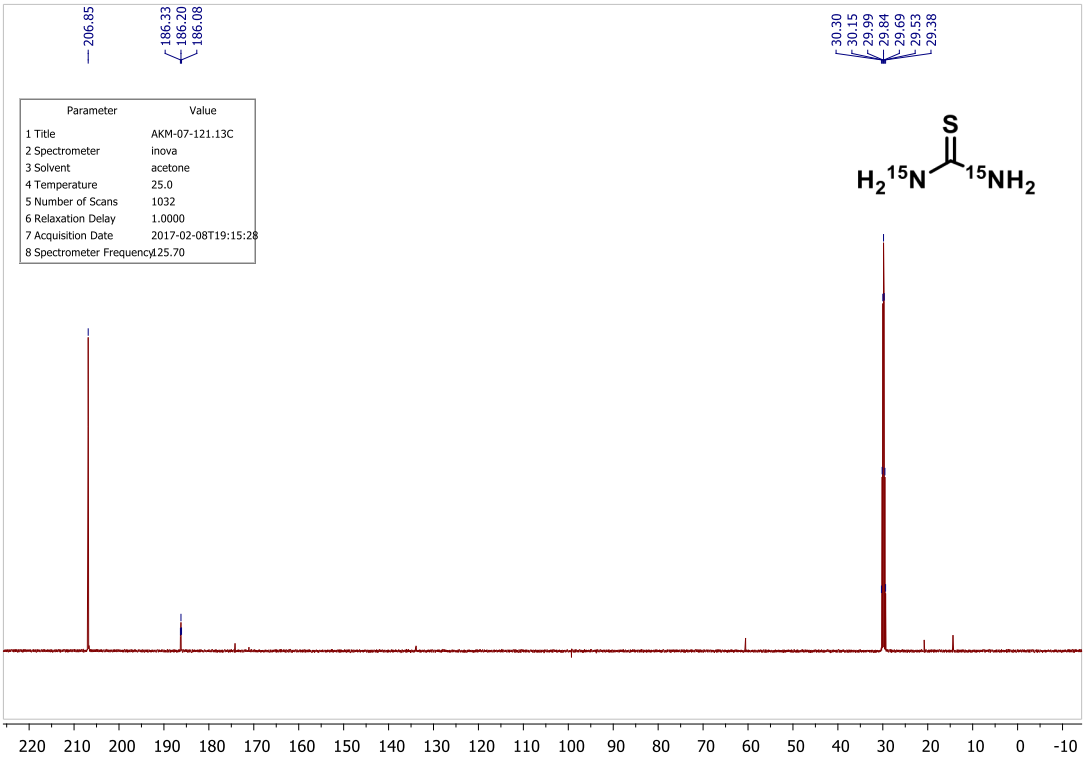

Parameter Value
1 Title AKM-07-98.15N
2 Spectrometer vnmrs
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 48
6 Relaxation Delay 2.0000
7 Acquisition Date 2017-02-01T12:27:08
8 Spectrometer Frequency 40.51
-145.47
-145.50
-165.62
-165.64
MeO
Br
15N
15N
OMe
70
-20 -40 -60 -80 -100 -130 -160 -190 -220 -250 -280 -310

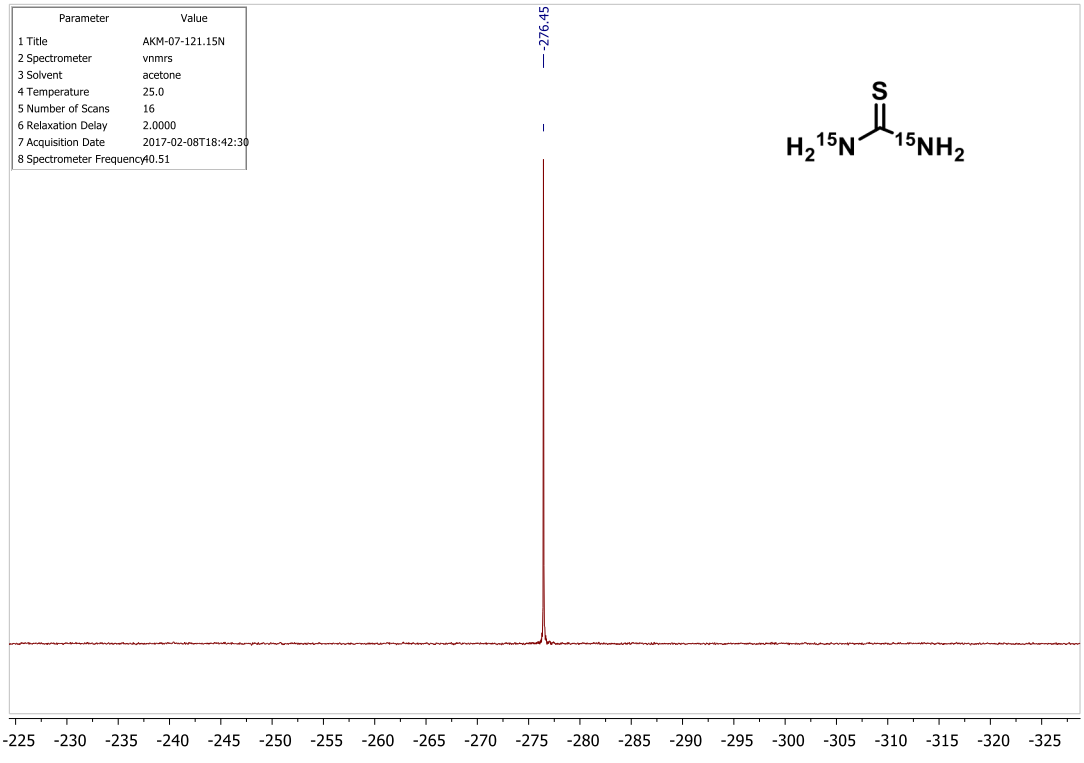

Parameter Value
1 Title AKM-07-101
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 30.0
5 Number of Scans 8
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-01-28T10:48:57
8 Spectrometer Frequency 499.86
7.49
7.48
7.48
7.47
7.46
7.36
7.34
7.25
7.25
7.24
7.24
7.23
S
PhO
OPh
2.06
1.00
2.02
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

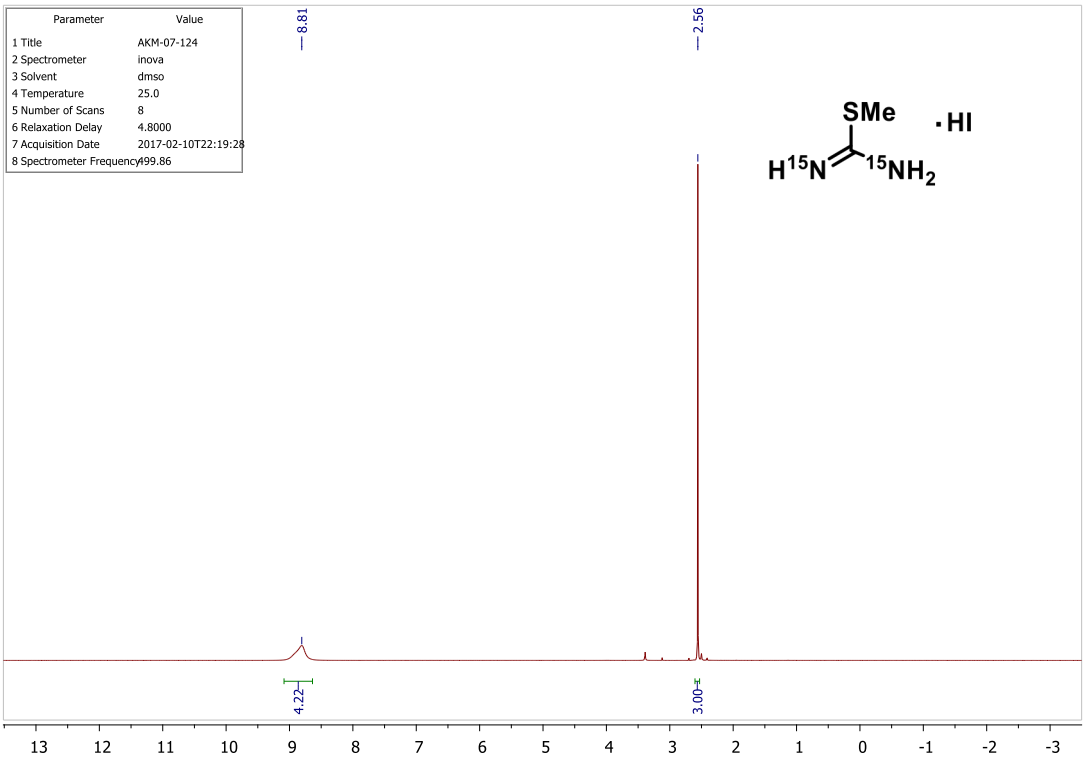

194.68
153.45
129.56
126.71
121.72
77.21
77.00
76.79
Parameter Value
1 Title AKM-07-81.13C
2 Spectrometer inova
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 28
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-09-04T15:31:35
8 Spectrometer Frequency 150.79
S
PhO
OPh
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

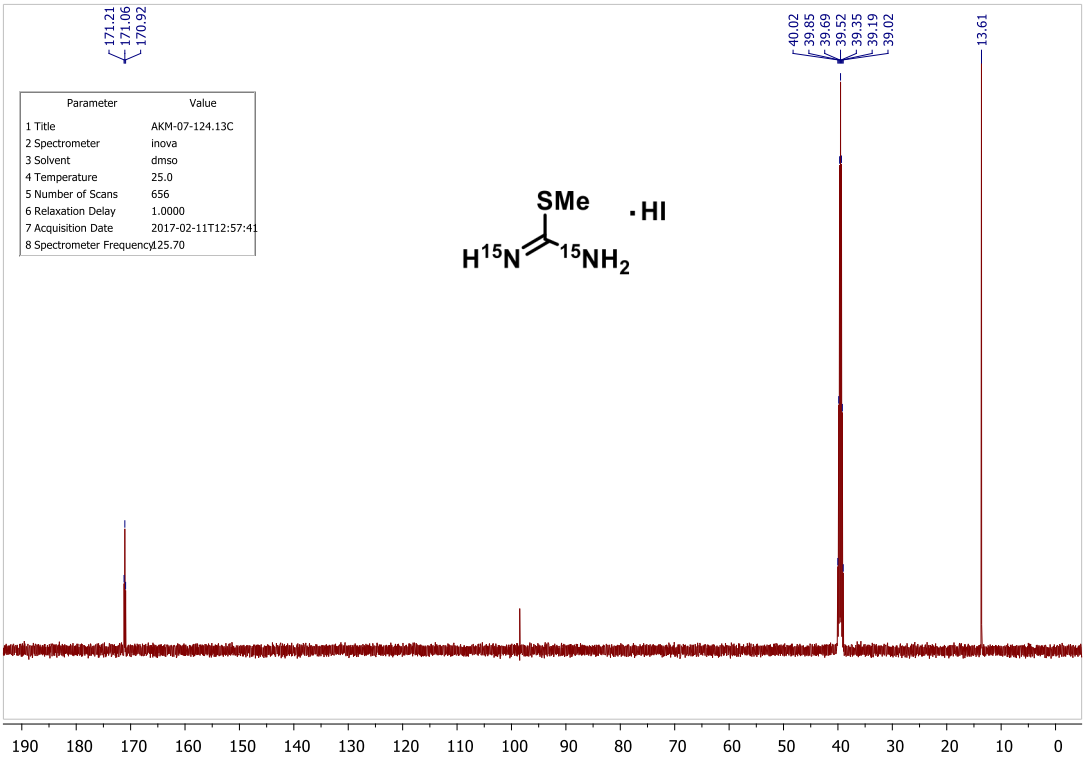

Parameter Value
1 Title AKM-07-111.13C
2 Spectrometer vnmrs
3 Solvent d2o
4 Temperature 25.0
5 Number of Scans 368
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-03-23T20:14:45
8 Spectrometer Frequency 100.54
133.57
133.43
15NH4SC15N
190 185 180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75

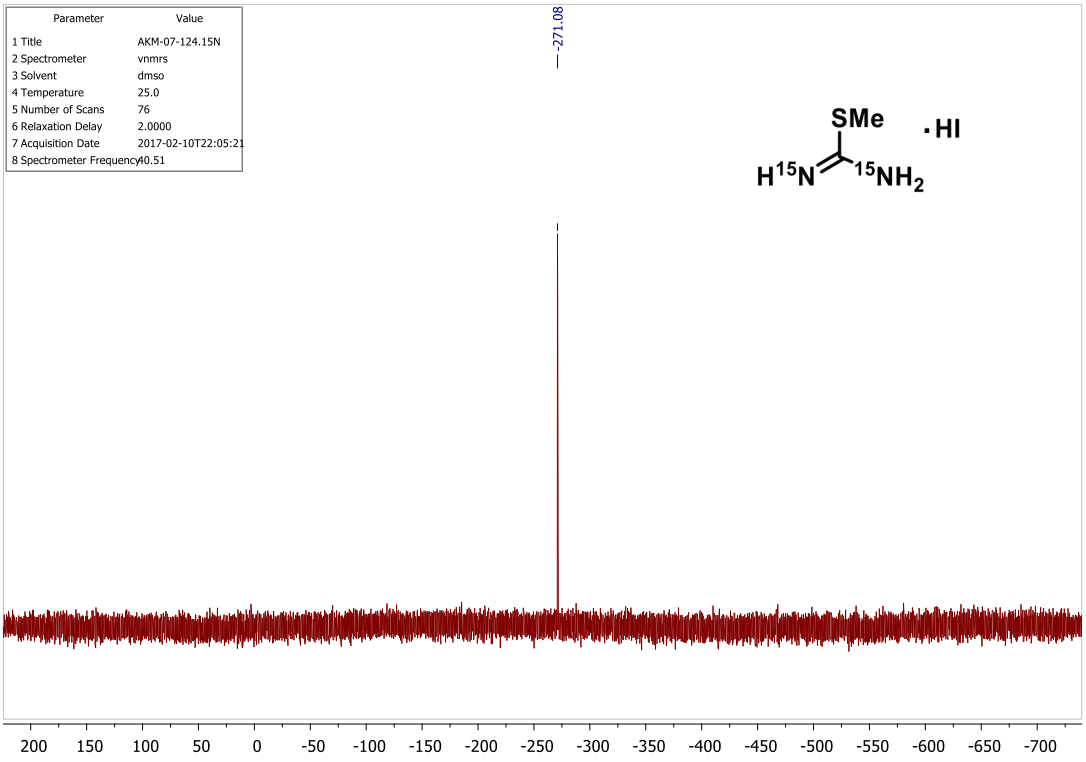

Parameter Value
1 Title AKM-07-111.15N
2 Spectrometer vnmrs
3 Solvent d2o
4 Temperature 25.0
5 Number of Scans 32
6 Relaxation Delay 2.0000
7 Acquisition Date 2017-03-23T20:07:49
8 Spectrometer Frequency 40.51
-174.65
15NH4SC15N
200 150 100 50 0 -50 -100 -150 -200 -250 -300 -350 -400 -450 -500 -550 -600

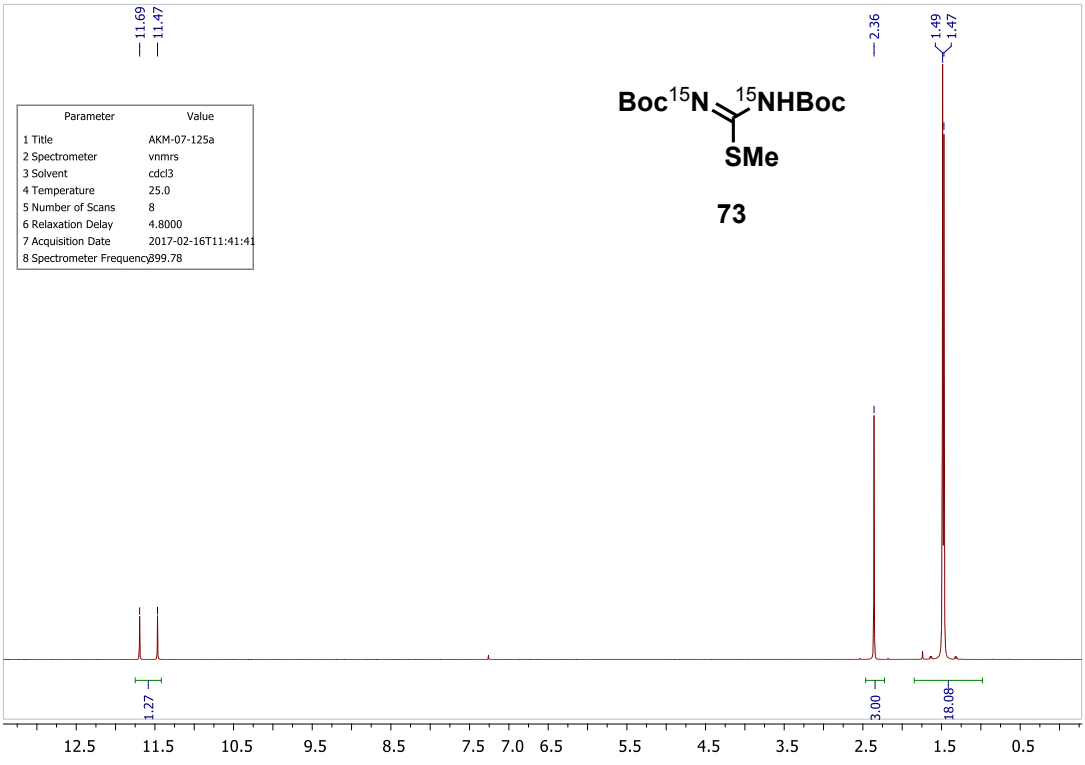

206.85
186.33
186.20
186.08
30.30
30.15
29.99
29.84
29.69
29.53
29.38
Parameter Value
1 Title AKM-07-121.13C
2 Spectrometer inova
3 Solvent acetone
4 Temperature 25.0
5 Number of Scans 1032
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-02-08T19:15:28
8 Spectrometer Frequency 125.70
S
H2 15N 15NH2
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10

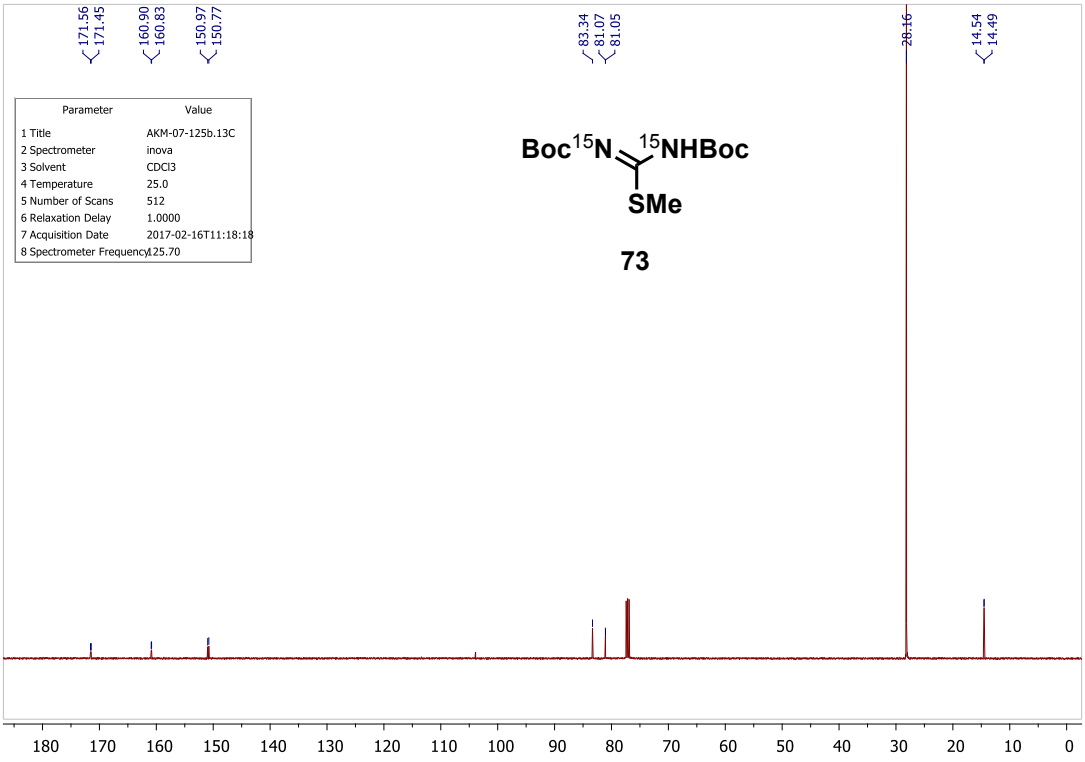

Parameter Value
1 Title AKM-07-121.15N
2 Spectrometer vnmrs
3 Solvent acetone
4 Temperature 25.0
5 Number of Scans 16
6 Relaxation Delay 2.0000
7 Acquisition Date 2017-02-08T18:42:30
8 Spectrometer Frequency 40.51
-276.45
S
H2 15N 15NH2
-225 -230 -235 -240 -245 -250 -255 -260 -265 -270 -275 -280 -285 -290 -295 -300 -305 -310 -315 -320 -325

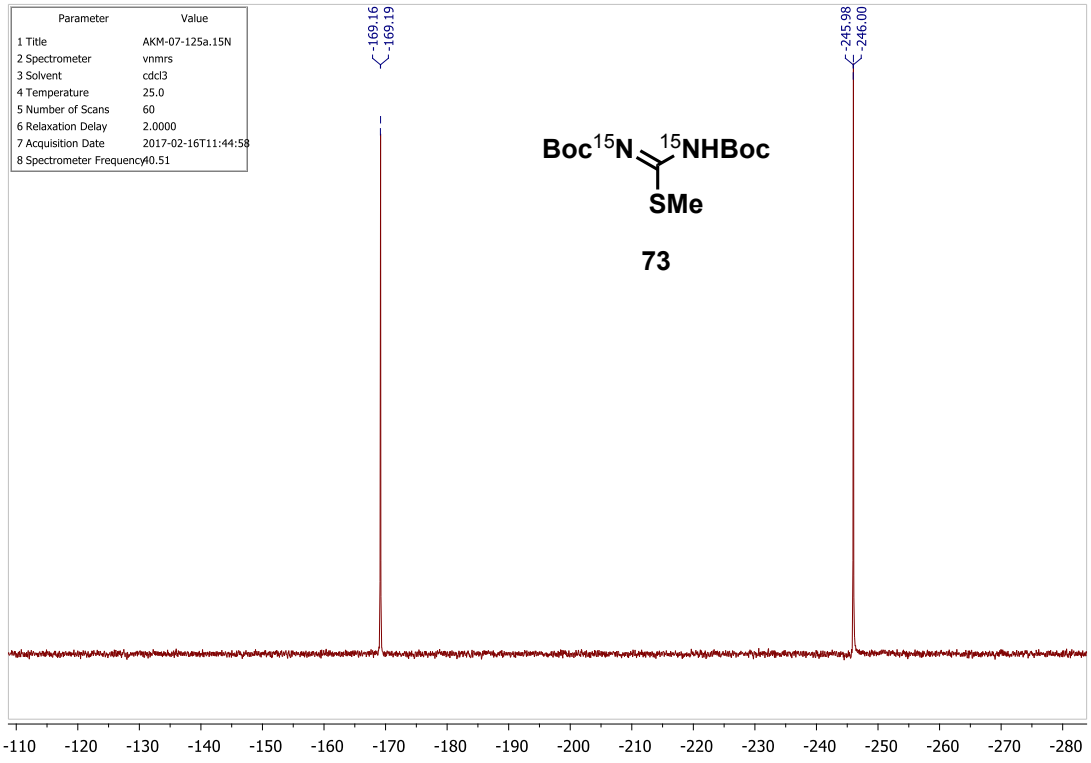

Parameter Value
1 Title AKM-07-124
2 Spectrometer inova
3 Solvent dmso
4 Temperature 25.0
5 Number of Scans 8
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-02-10T22:19:28
8 Spectrometer Frequency 499.86
8.81
2.56
SMe
·HI
H15N 15NH2
4.22
3.00
13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3

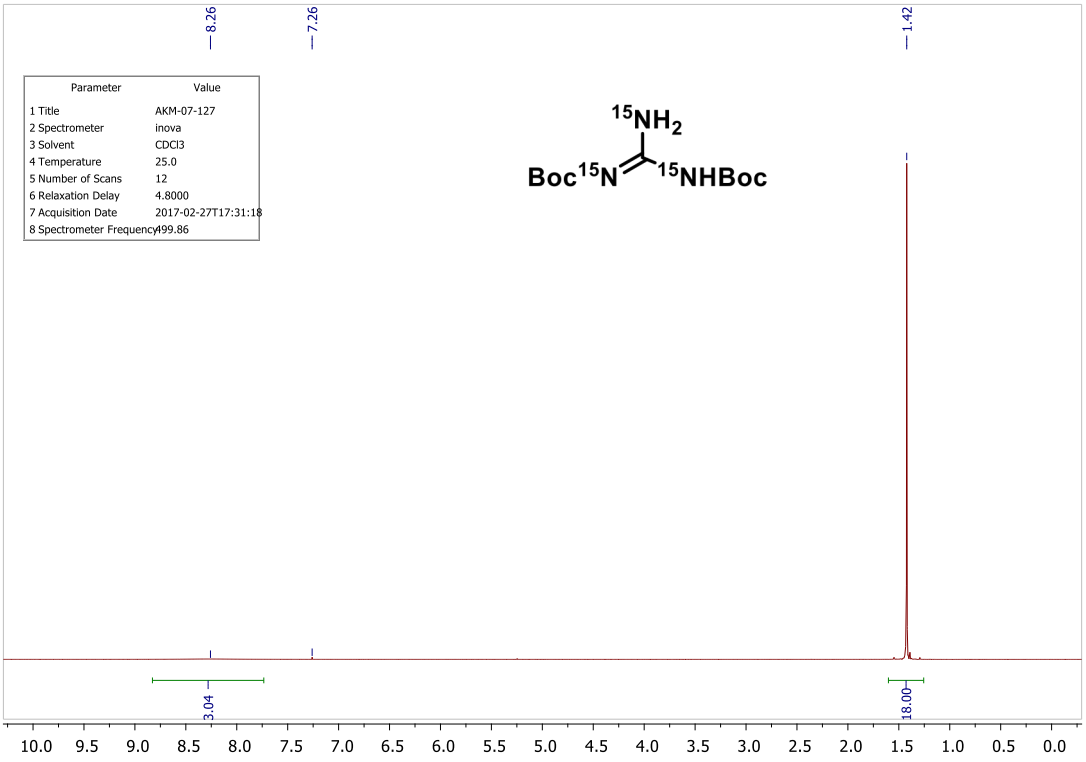

171.21
171.06
170.92
40.02
39.85
39.69
39.52
39.35
39.19
39.02
13.61
Parameter Value
1 Title AKM-07-124.13C
2 Spectrometer inova
3 Solvent dmso
4 Temperature 25.0
5 Number of Scans 656
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-02-11T12:57:41
8 Spectrometer Frequency 125.70
SMe
·HI
H15N
15NH2
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

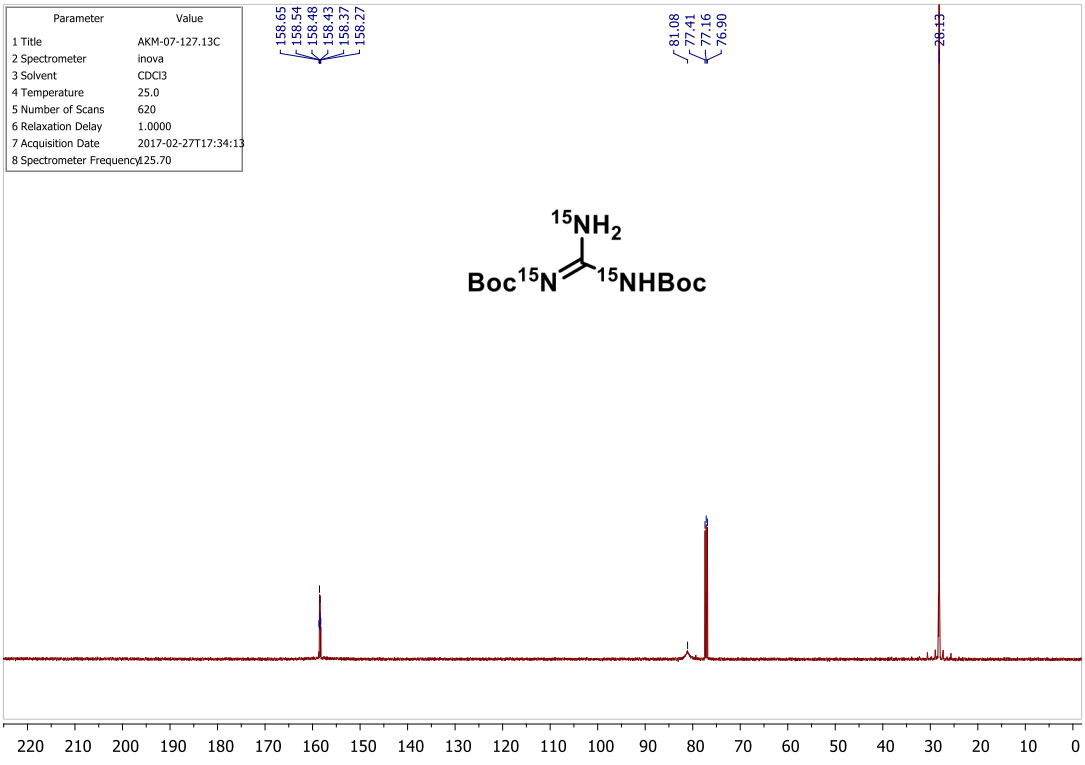

-271.08
Parameter Value
1 Title AKM-07-124.15N
2 Spectrometer vnmrs
3 Solvent dmso
4 Temperature 25.0
5 Number of Scans 76
6 Relaxation Delay 2.0000
7 Acquisition Date 2017-02-10T22:05:21
8 Spectrometer Frequency 40.51
SMe
·HI
H15N
15NH2
200 150 100 50 0 -50 -100 -150 -200 -250 -300 -350 -400 -450 -500 -550 -600 -650 -700

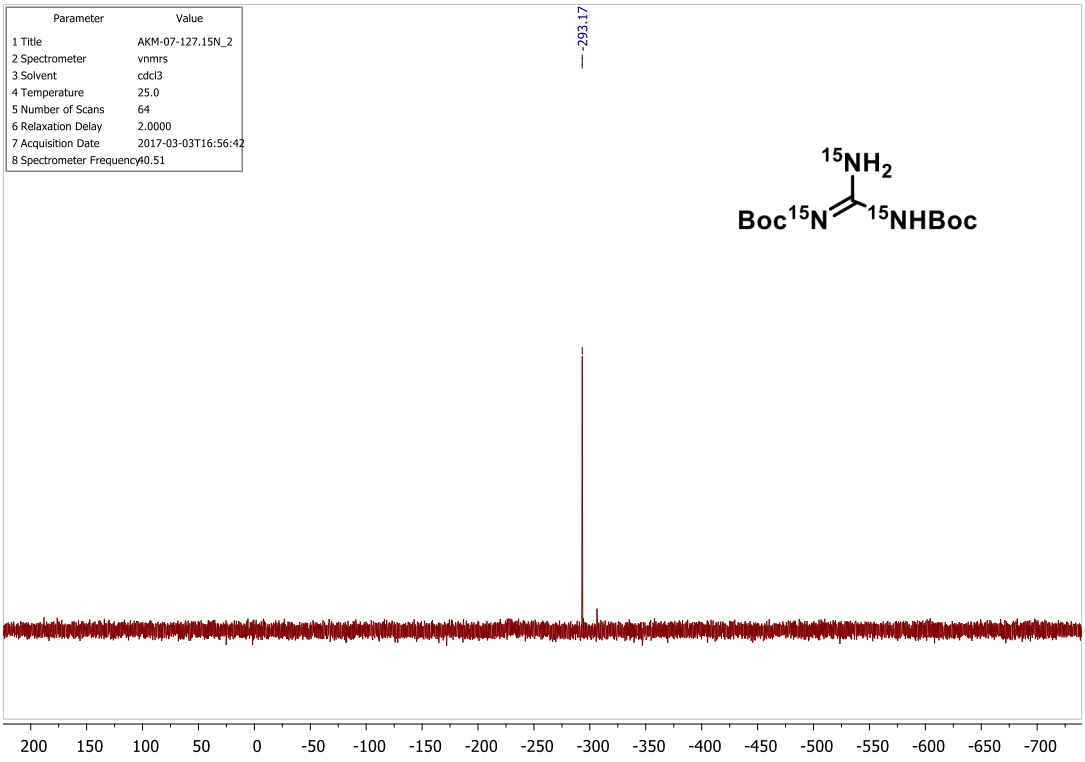

11.69
11.47
2.36
1.49
1.47
Parameter Value
1 Title AKM-07-125a
2 Spectrometer vnmrs
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 8
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-02-16T11:41:41
8 Spectrometer Frequency 399.78
Boc15N 15NHBoc
SMe
73
1.27
3.00
18.08
12.5 11.5 10.5 9.5 8.5 7.5 7.0 6.5 5.5 4.5 3.5 2.5 1.5 0.5

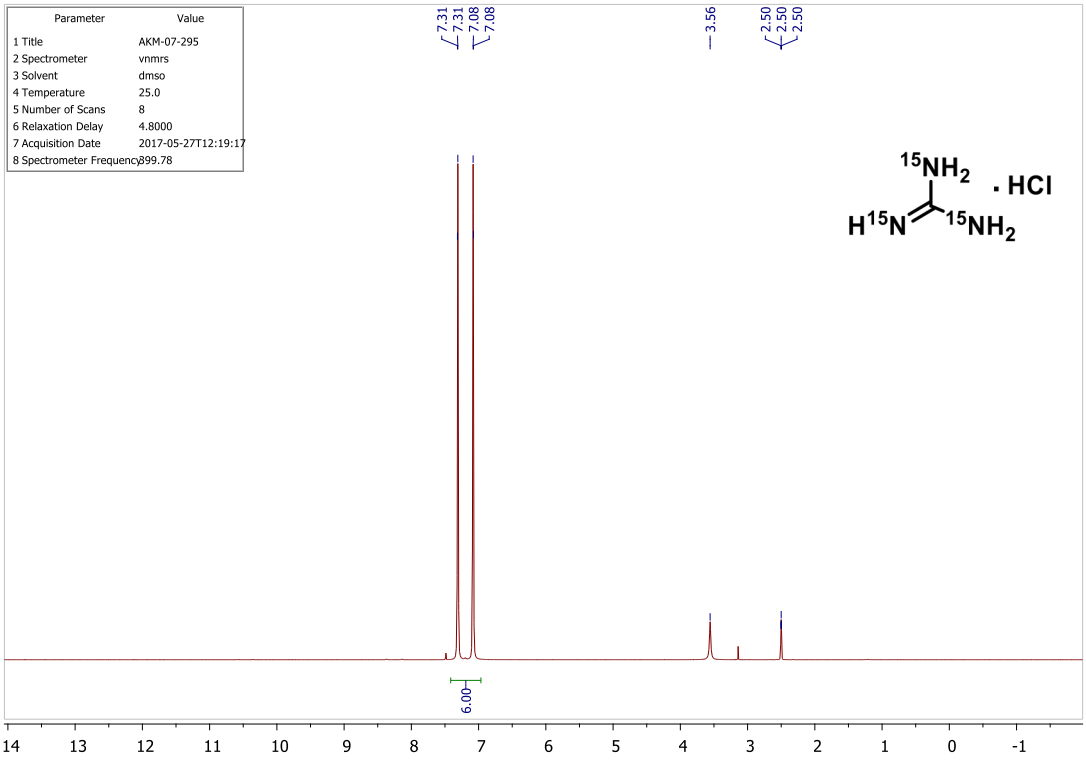

171.56
171.45
160.90
160.83
150.97
150.77
83.34
81.07
81.05
28.16
14.54
14.49
Parameter Value
1 Title AKM-07-125b.13C
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 25.0
5 Number of Scans 512
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-02-16T11:18:18
8 Spectrometer Frequency 125.70
Boc15N 15NHBoc
SMe
73
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

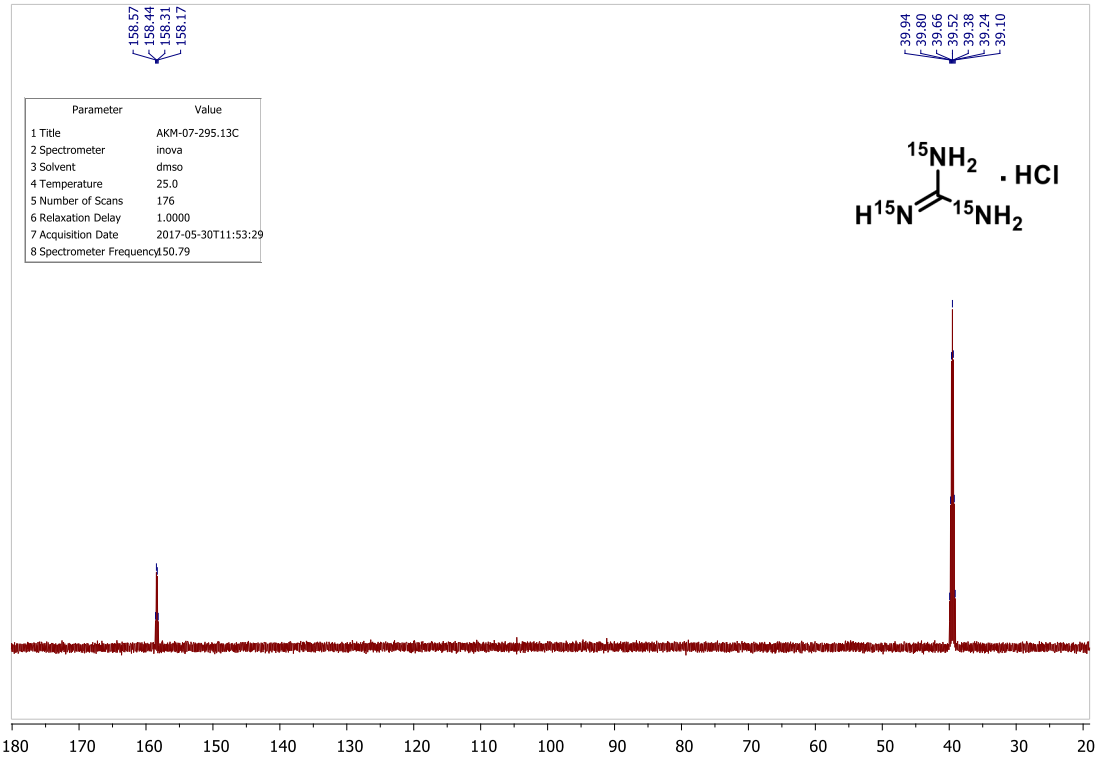

Parameter Value
1 Title AKM-07-125a.15N
2 Spectrometer vnmrs
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 60
6 Relaxation Delay 2.0000
7 Acquisition Date 2017-02-16T11:44:58
8 Spectrometer Frequency 40.51
-169.16
-169.19
-245.98
-246.00
Boc15N 15NHBoc
SMe
73
-110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210 -220 -230 -240 -250 -260 -270 -280

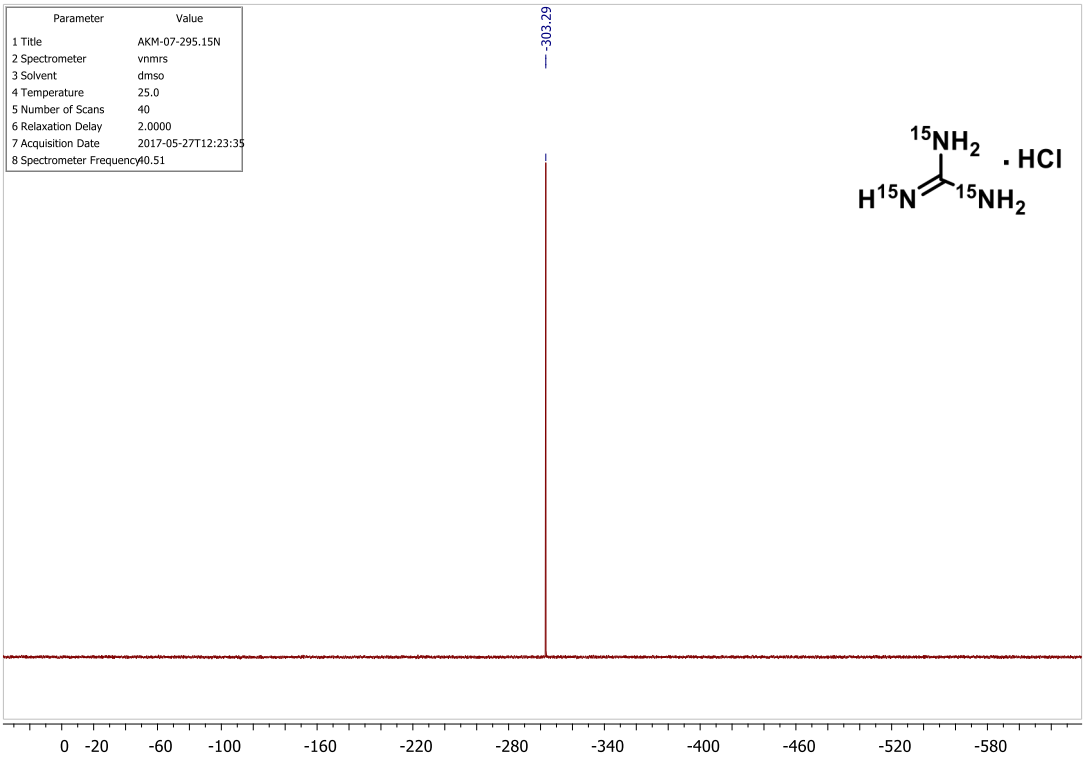

8.26
7.26
1.42
Parameter Value
1 Title AKM-07-127
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 25.0
5 Number of Scans 12
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-02-27T17:31:18
8 Spectrometer Frequency 499.86
15NH2
Boc15N 15NHBoc
3.04
18.00
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

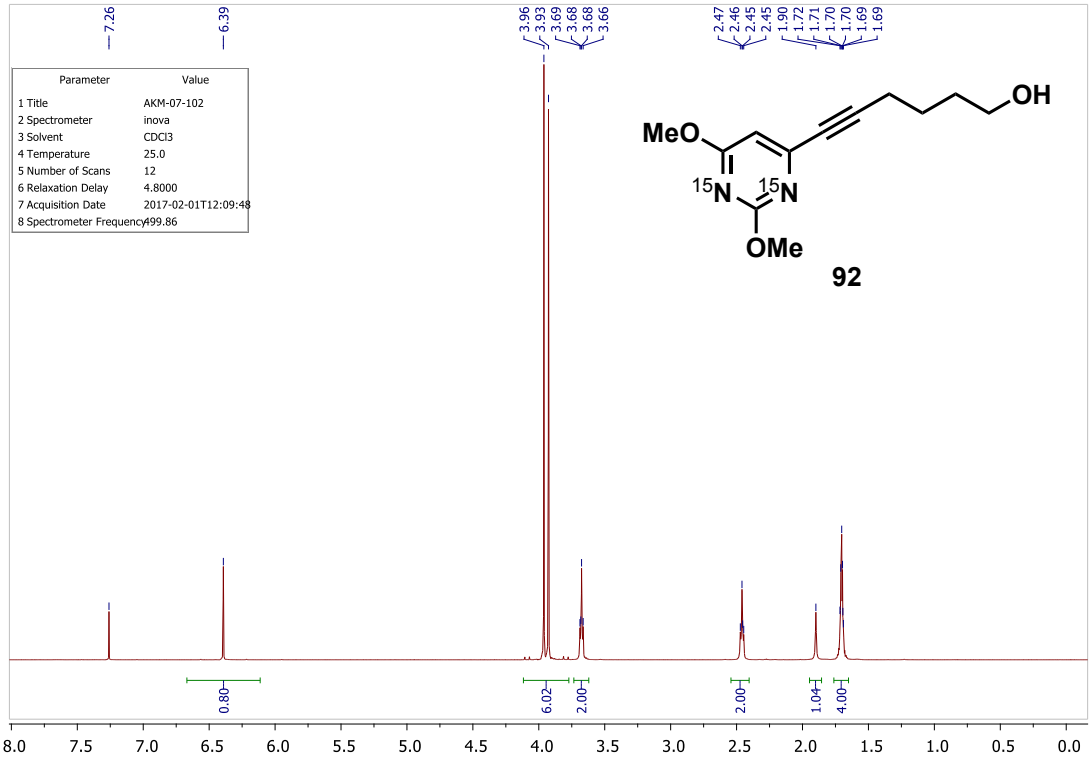

Parameter Value
1 Title AKM-07-127.13C
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 25.0
5 Number of Scans 620
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-02-27T17:34:13
8 Spectrometer Frequency 125.70
158.65
158.54
158.48
158.43
158.37
158.27
81.08
77.41
77.16
76.90
28.13
15NH2
Boc15N
15NHBoc
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

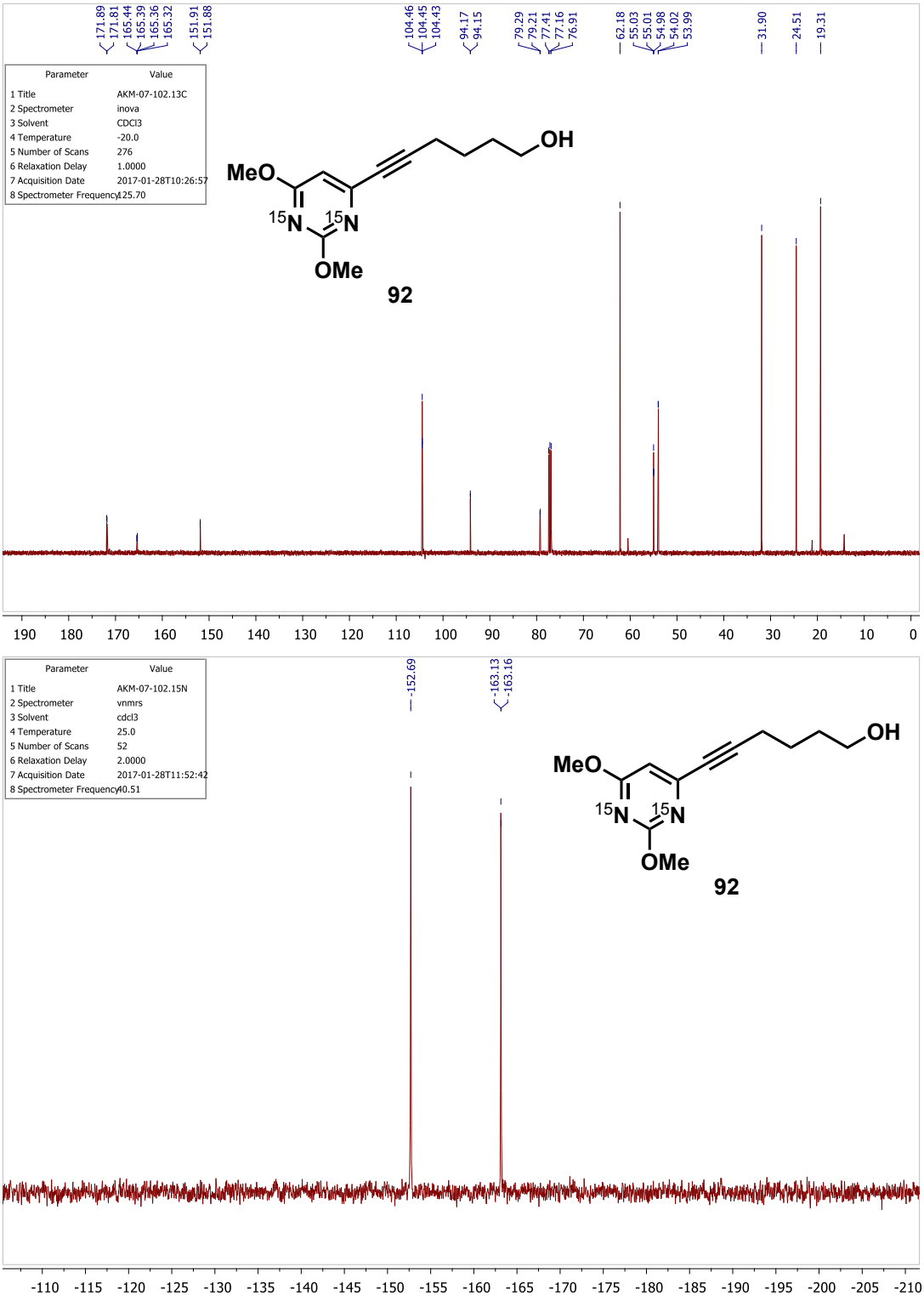

Parameter Value
1 Title AKM-07-127.15N_2
2 Spectrometer vnmrs
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 64
6 Relaxation Delay 2.0000
7 Acquisition Date 2017-03-03T16:56:42
8 Spectrometer Frequency 40.51
-293.17
15NH2
Boc15N
15NHBoc
200 150 100 50 0 -50 -100 -150 -200 -250 -300 -350 -400 -450 -500 -550 -600 -650 -700

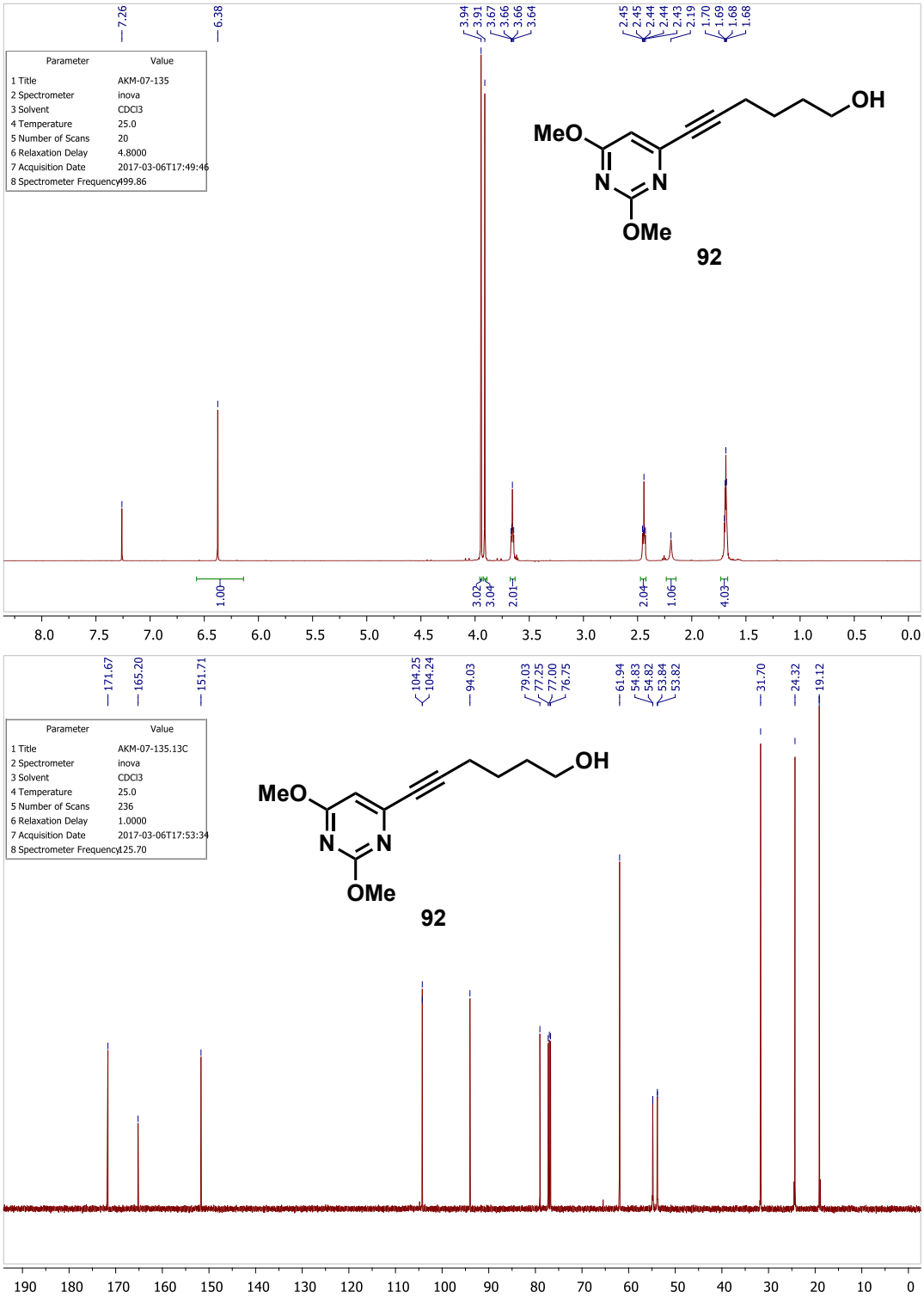

Parameter Value
1 Title AKM-07-295
2 Spectrometer vnmrs
3 Solvent dmso
4 Temperature 25.0
5 Number of Scans 8
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-05-27T12:19:17
8 Spectrometer Frequency 399.78
7.31
7.31
7.08
7.08
3.56
2.50
2.50
2.50
6.00
$^{15}NH_2$
$H^{15}N$
$^{15}NH_2$
· HCl
14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1

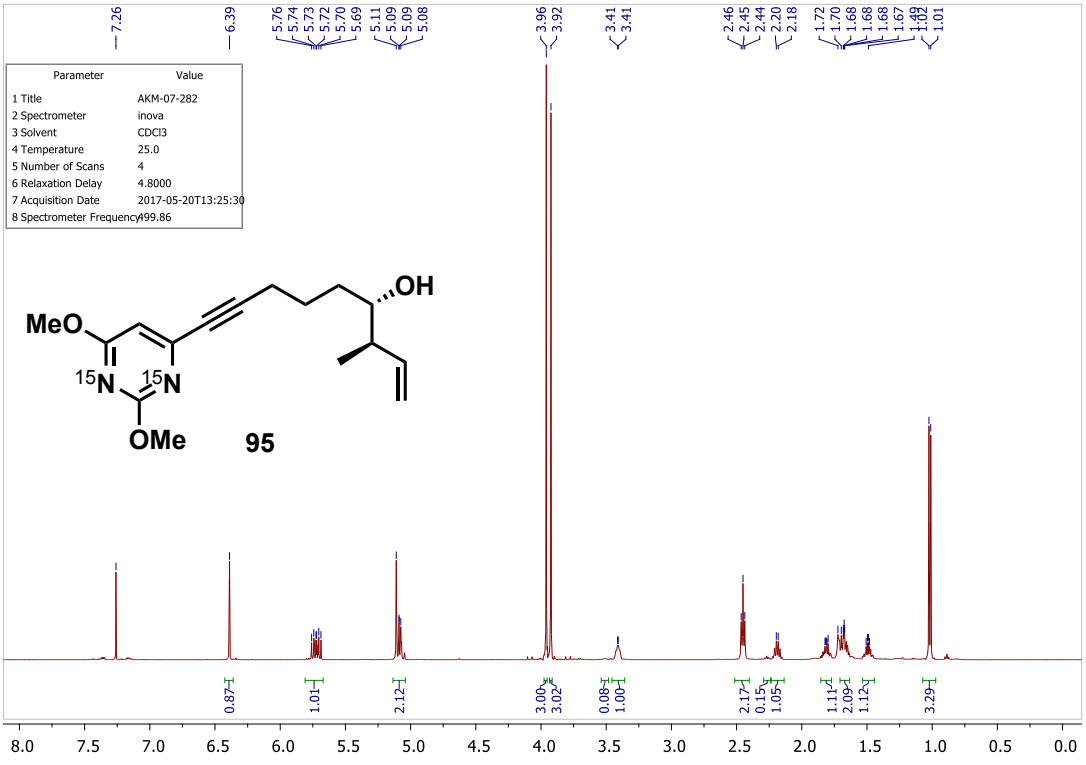

158.57
158.44
158.31
158.17
39.94
39.80
39.66
39.52
39.38
39.24
39.10
Parameter Value
1 Title AKM-07-295.13C
2 Spectrometer inova
3 Solvent dmso
4 Temperature 25.0
5 Number of Scans 176
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-05-30T11:53:29
8 Spectrometer Frequency 150.79
$^{15}NH_2$
$H^{15}N$
$^{15}NH_2$
· HCl
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20

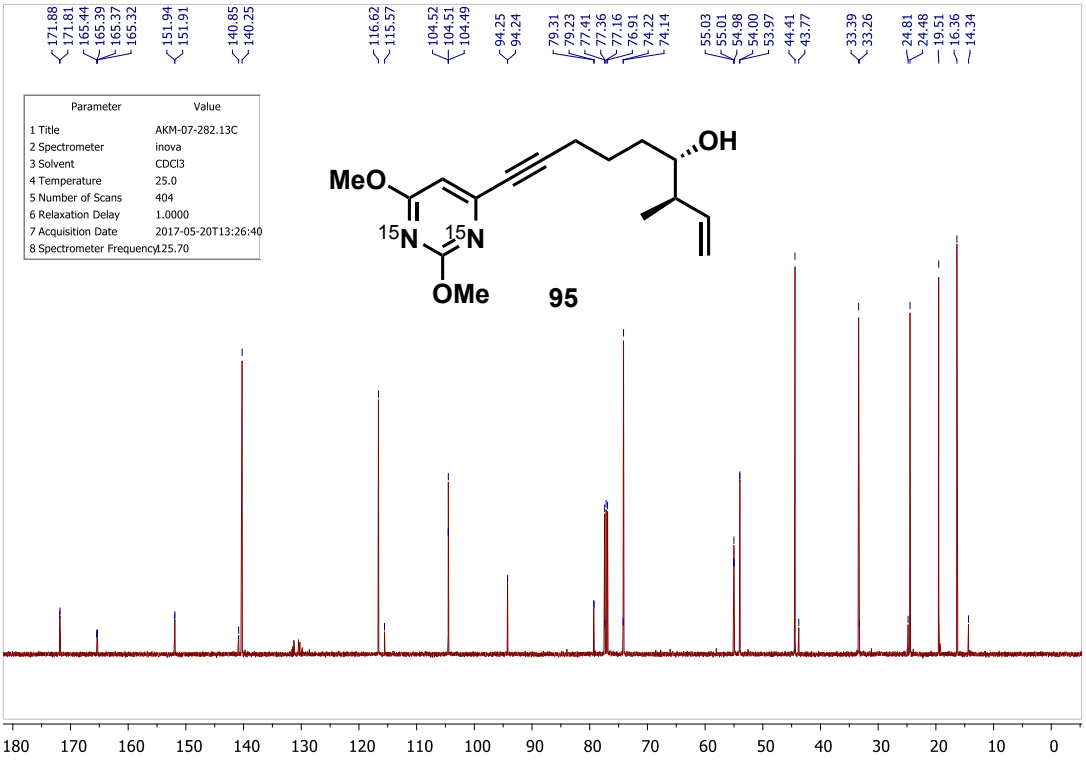

Parameter Value
1 Title AKM-07-295.15N
2 Spectrometer vnmrs
3 Solvent dmso
4 Temperature 25.0
5 Number of Scans 40
6 Relaxation Delay 2.0000
7 Acquisition Date 2017-05-27T12:23:35
8 Spectrometer Frequency 40.51
-303.29
15NH2
H15N
15NH2
· HCl
0 -20 -60 -100 -160 -220 -280 -340 -400 -460 -520 -580

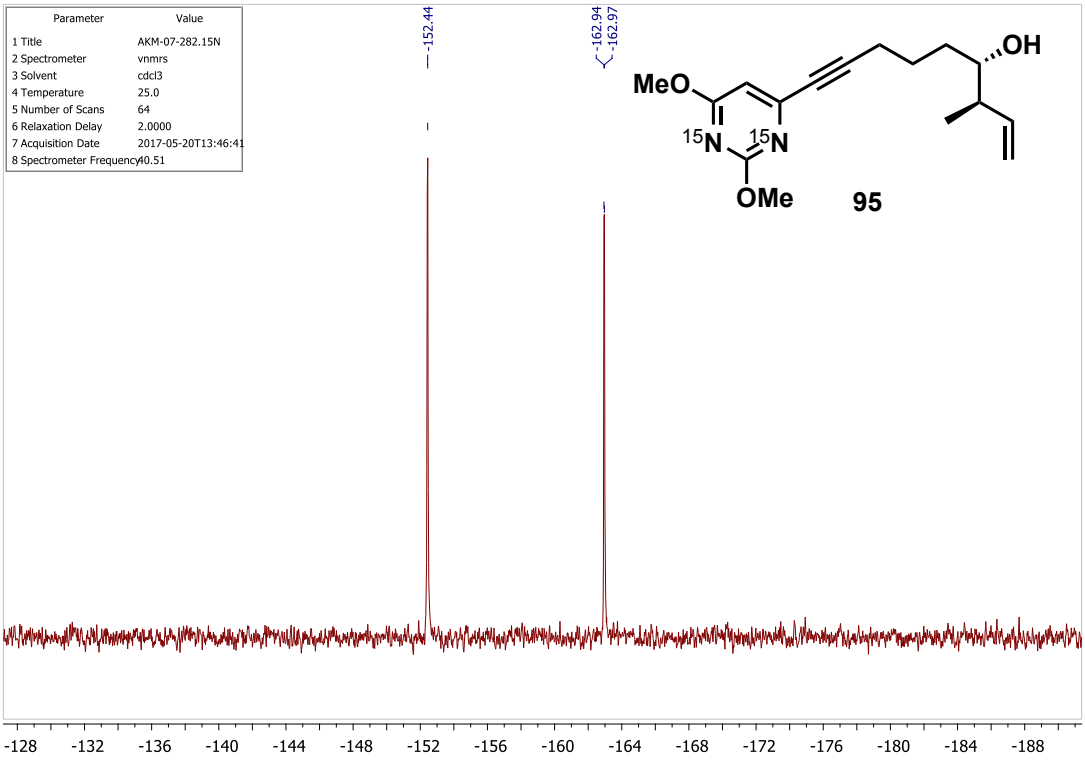

Parameter Value
1 Title AKM-07-102
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 25.0
5 Number of Scans 12
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-02-01T12:09:48
8 Spectrometer Frequency 499.86
7.26
6.39
3.96 3.93 3.69 3.68 3.68 3.66
2.47 2.46 2.45 2.45 1.90 1.72 1.71 1.70 1.70 1.69 1.69
MeO
OH
15N
15N
OMe
92
0.80
6.02
2.00
2.00
1.04
4.00
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

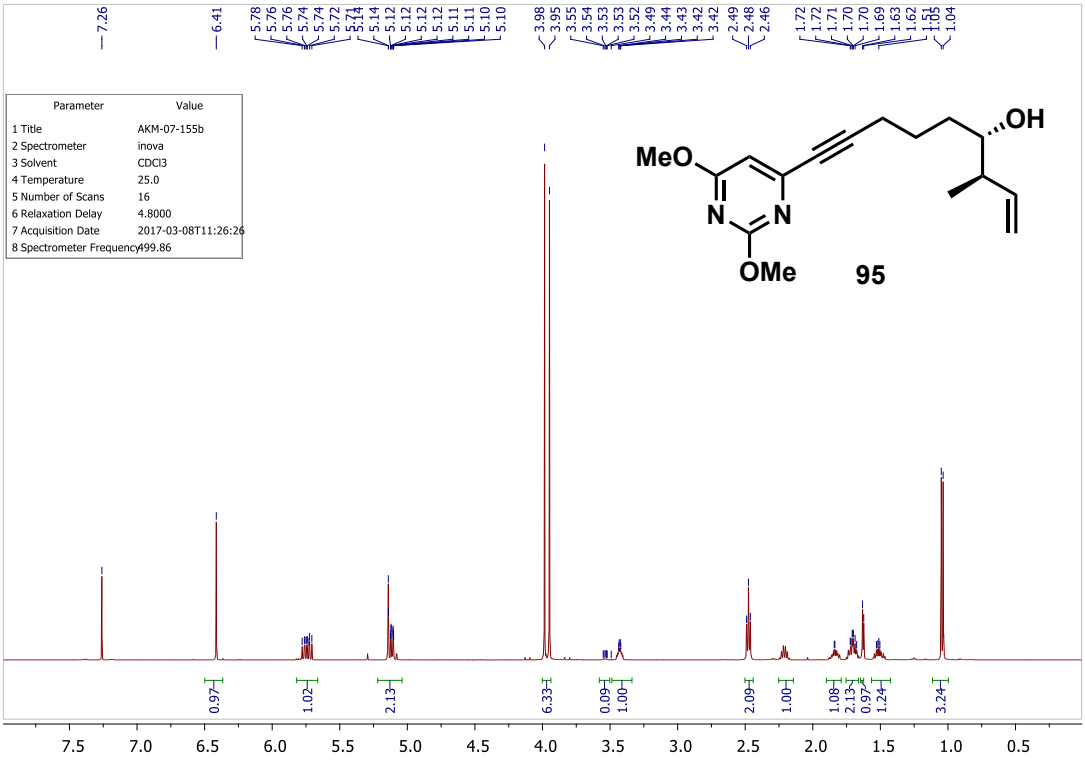

171.89
171.81
165.44
165.39
165.36
165.32
151.91
151.88
104.46
104.45
104.43
94.17
94.15
79.29
79.21
77.41
77.16
76.91
62.18
55.03
55.01
54.98
54.02
53.99
31.90
24.51
19.31
Parameter Value
1 Title AKM-07-102.13C
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature -20.0
5 Number of Scans 276
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-01-28T10:26:57
8 Spectrometer Frequency 125.70
MeO
OH
15N
15N
OMe
92
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
Parameter Value
1 Title AKM-07-102.15N
2 Spectrometer vnmrs
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 52
6 Relaxation Delay 2.0000
7 Acquisition Date 2017-01-28T11:52:42
8 Spectrometer Frequency 40.51
-152.69
-163.13
-163.16
MeO
OH
15N
15N
OMe
92
-110 -115 -120 -125 -130 -135 -140 -145 -150 -155 -160 -165 -170 -175 -180 -185 -190 -195 -200 -205 -210

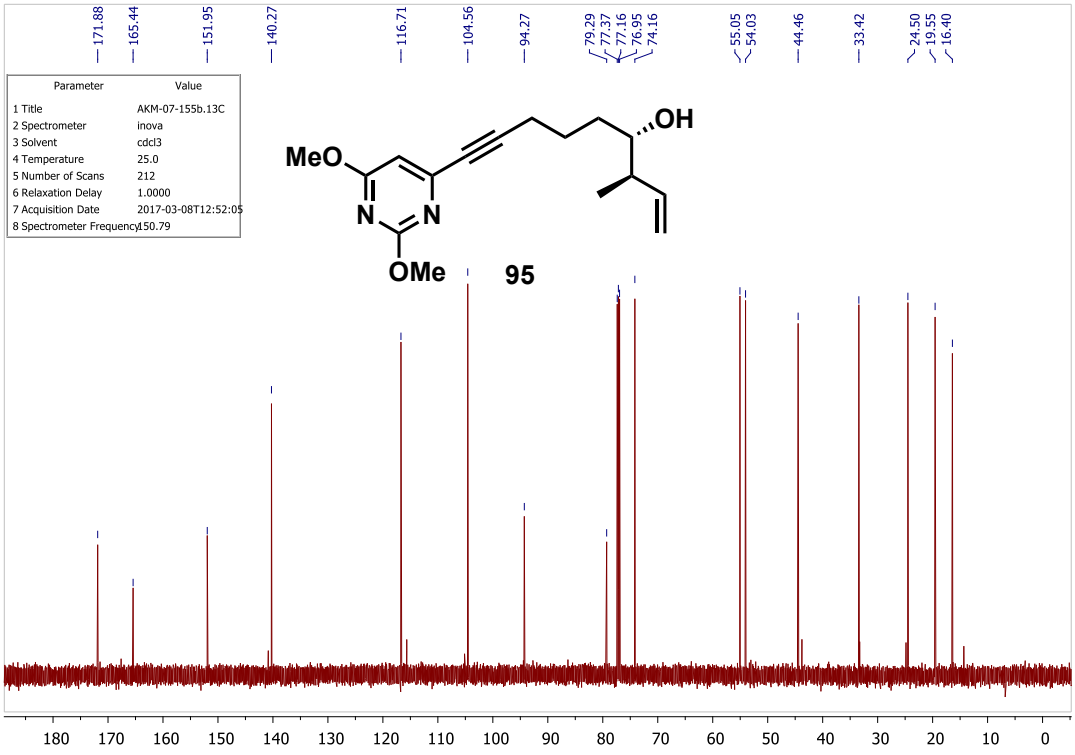

7.26
6.38
3.94
3.91
3.67
3.66
3.66
3.64
2.45
2.45
2.44
2.44
2.43
2.19
1.70
1.69
1.68
1.68
Parameter Value
1 Title AKM-07-135
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 25.0
5 Number of Scans 20
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-03-06T17:49:46
8 Spectrometer Frequency 499.86
MeO
OH
N
N
OMe
92
1.00
3.02
3.04
2.01
2.04
1.06
4.03
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
171.67
165.20
151.71
104.25
104.24
94.03
79.03
77.25
77.00
76.75
61.94
54.83
54.82
53.84
53.82
31.70
24.32
19.12
Parameter Value
1 Title AKM-07-135.13C
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 25.0
5 Number of Scans 236
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-03-06T17:53:34
8 Spectrometer Frequency 125.70
MeO
OH
N
N
OMe
92
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

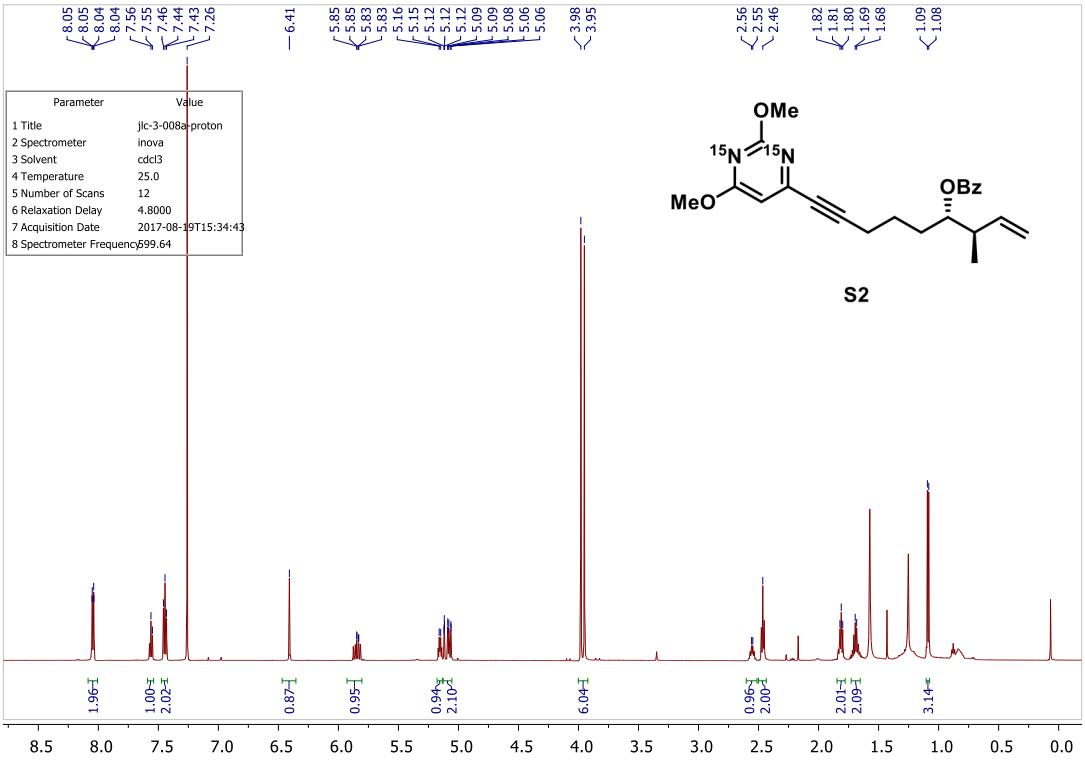

7.26
6.39
5.76
5.74
5.73
5.72
5.70
5.69
5.11
5.09
5.09
5.08
3.96
3.92
3.41
3.41
2.46
2.45
2.44
2.20
2.18
1.72
1.70
1.68
1.68
1.68
1.67
1.49
1.02
1.01
Parameter Value
1 Title AKM-07-282
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 25.0
5 Number of Scans 4
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-05-20T13:25:30
8 Spectrometer Frequency 499.86
MeO
OH
15N
15N
OMe
95
0.87
1.01
2.12
3.00
3.02
0.08
1.00
2.17
0.15
1.05
1.11
2.09
1.12
3.29
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

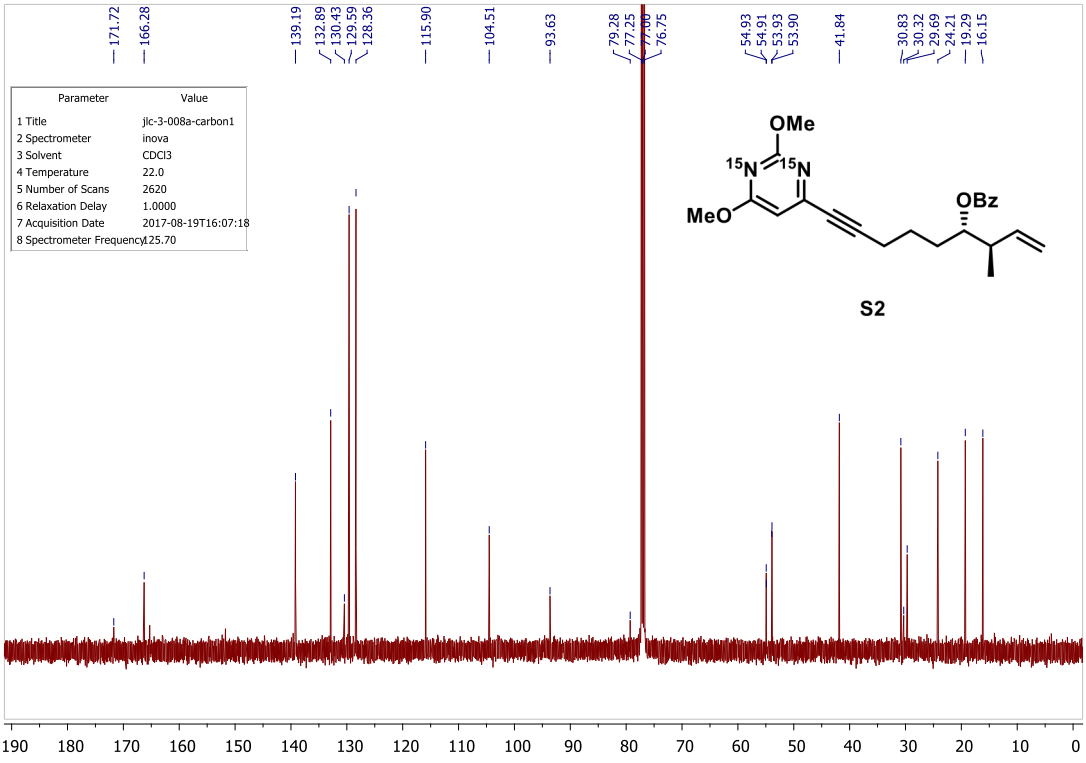

171.88
171.81
165.44
165.39
165.37
165.32
151.94
151.91
140.85
140.25
116.62
115.57
104.52
104.51
104.49
94.25
94.24
79.31
79.23
77.41
77.36
77.16
76.91
74.22
74.14
55.03
55.01
54.98
54.00
53.97
44.41
43.77
33.39
33.26
24.81
24.48
19.51
16.36
14.34
Parameter Value
1 Title AKM-07-282.13C
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 25.0
5 Number of Scans 404
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-05-20T13:26:40
8 Spectrometer Frequency 125.70
MeO
OH
15N
15N
OMe
95
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

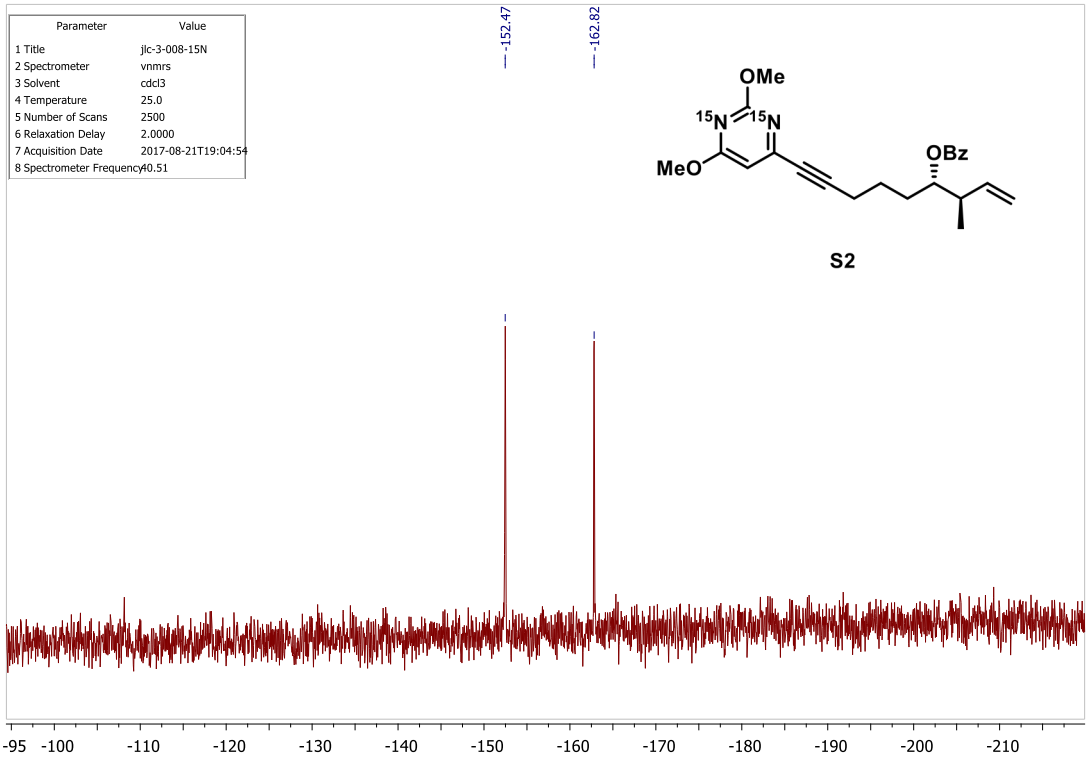

Parameter Value
1 Title AKM-07-282.15N
2 Spectrometer vnmrs
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 64
6 Relaxation Delay 2.0000
7 Acquisition Date 2017-05-20T13:46:41
8 Spectrometer Frequency 40.51
-152.44
-162.94
-162.97
MeO
15N
15N
OMe
OH
95
-128 -132 -136 -140 -144 -148 -152 -156 -160 -164 -168 -172 -176 -180 -184 -188

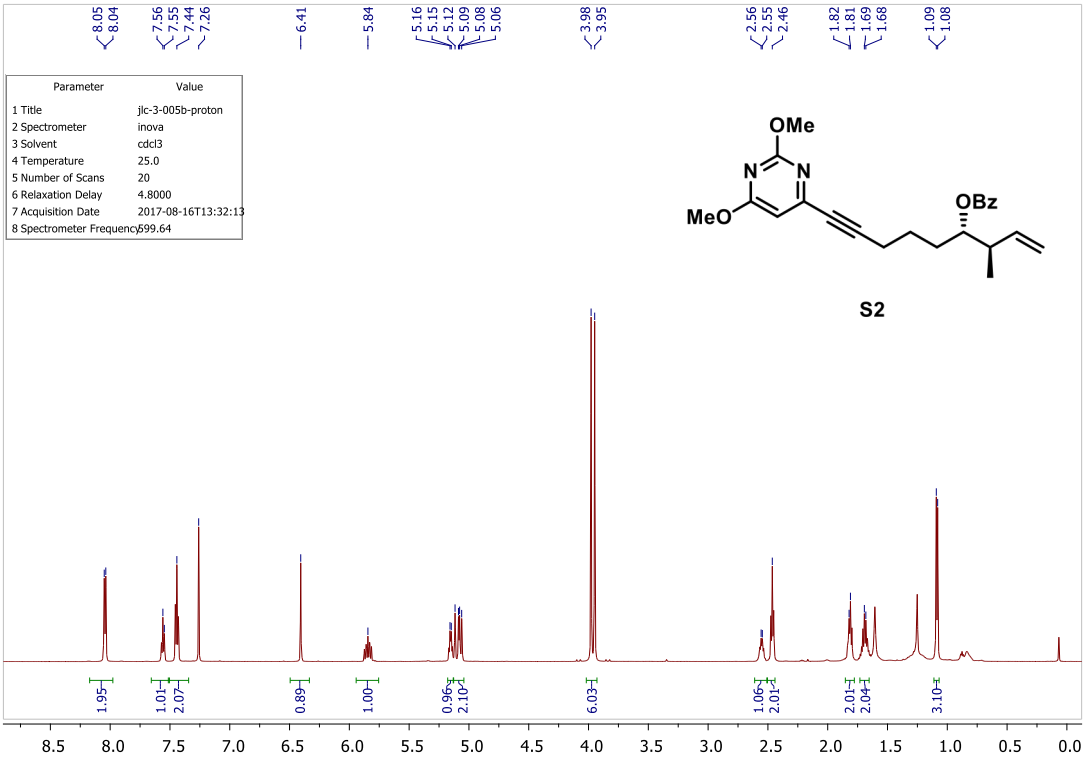

7.26
6.41
5.78 5.76 5.76 5.74 5.74 5.72 5.14 5.12 5.12 5.12 5.11 5.11 5.10 5.10
3.98 3.95 3.55 3.54 3.53 3.53 3.52 3.49 3.44 3.43 3.42 3.42
2.49 2.48 2.46
1.72 1.72 1.71 1.70 1.70 1.69 1.63 1.62 1.51 1.05 1.04
Parameter Value
1 Title AKM-07-155b
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 25.0
5 Number of Scans 16
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-03-08T11:26:26
8 Spectrometer Frequency 499.86
MeO
N
N
OMe
OH
95
0.97 1.02 2.13 6.33 0.09 1.00 2.09 1.00 1.08 2.13 0.97 1.24 3.24
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5

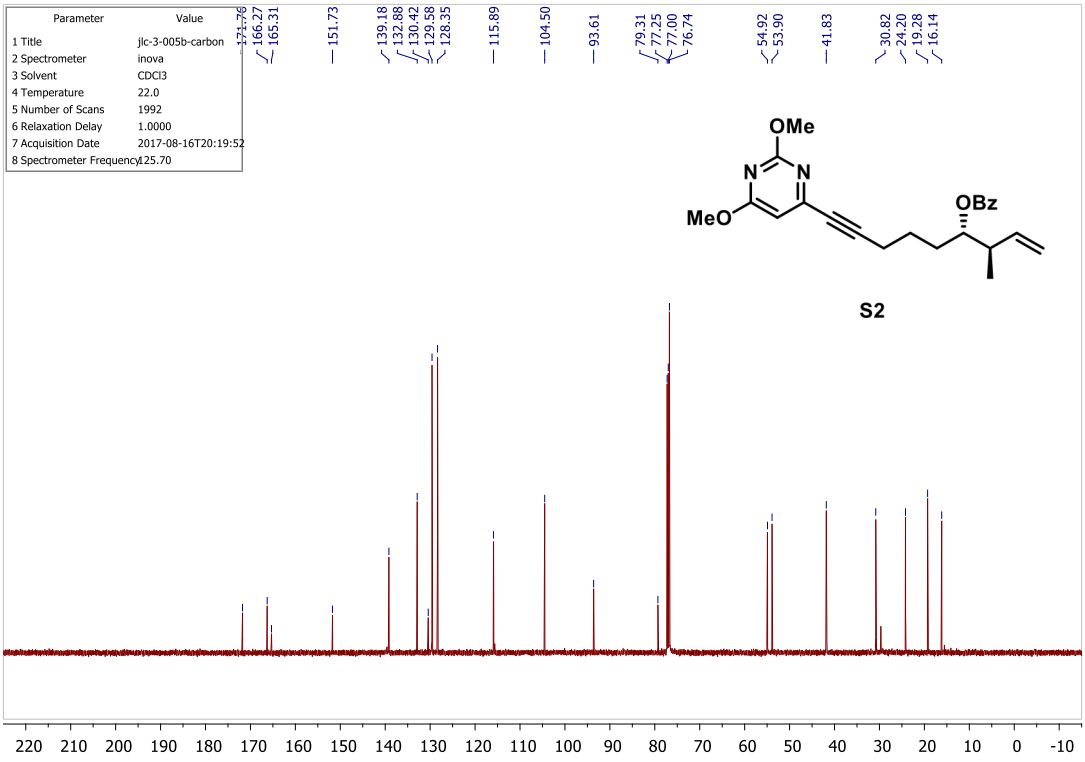

171.88
165.44
151.95
140.27
116.71
104.56
94.27
79.29
77.37
77.16
76.95
74.16
55.05
54.03
44.46
33.42
24.50
19.55
16.40
Parameter Value
1 Title AKM-07-155b.13C
2 Spectrometer inova
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 212
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-03-08T12:52:05
8 Spectrometer Frequency 150.79
MeO
OH
N
N
OMe
95
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

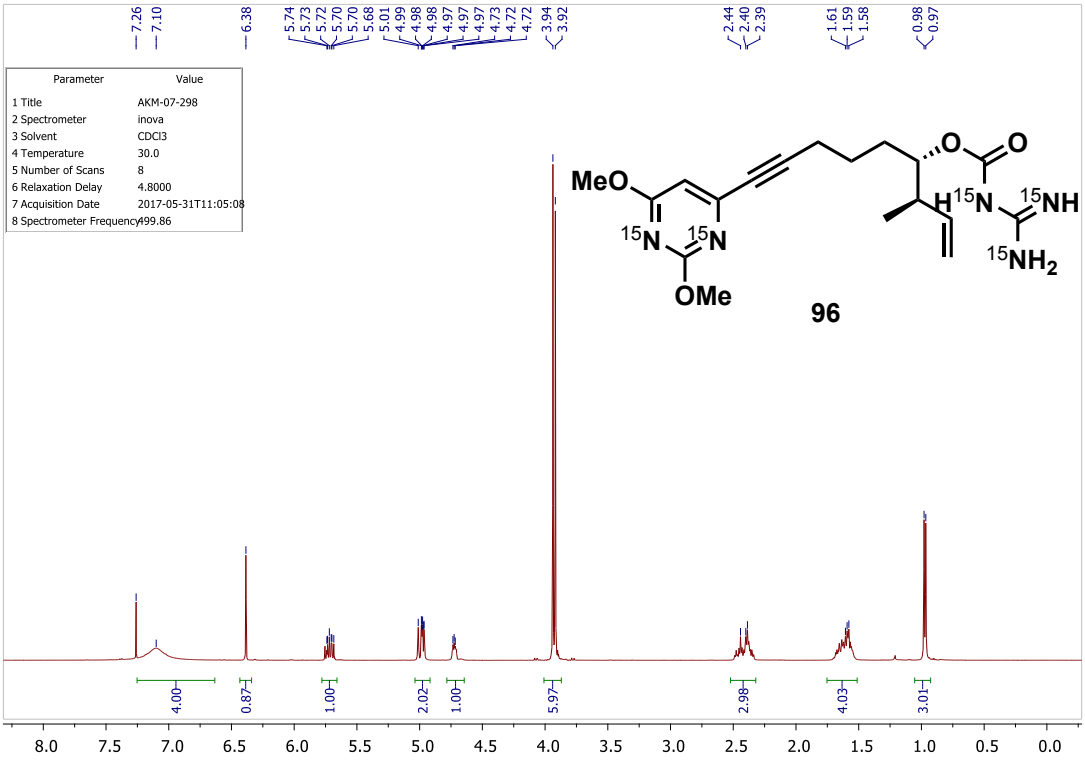

8.05
8.05
8.04
8.04
7.56
7.55
7.46
7.44
7.43
7.26
6.41
5.85
5.85
5.83
5.83
5.16
5.15
5.12
5.12
5.12
5.09
5.09
5.08
5.06
5.06
3.98
3.95
2.56
2.55
2.46
1.82
1.81
1.80
1.69
1.68
1.09
1.08
Parameter Value
1 Title jlc-3-008a-proton
2 Spectrometer inova
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 12
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-08-19T15:34:43
8 Spectrometer Frequency 599.64
OMe
15N
15N
MeO
OBz
S2
1.96
1.00
2.02
0.87
0.95
0.94
2.10
6.04
0.96
2.00
2.01
2.09
3.14
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

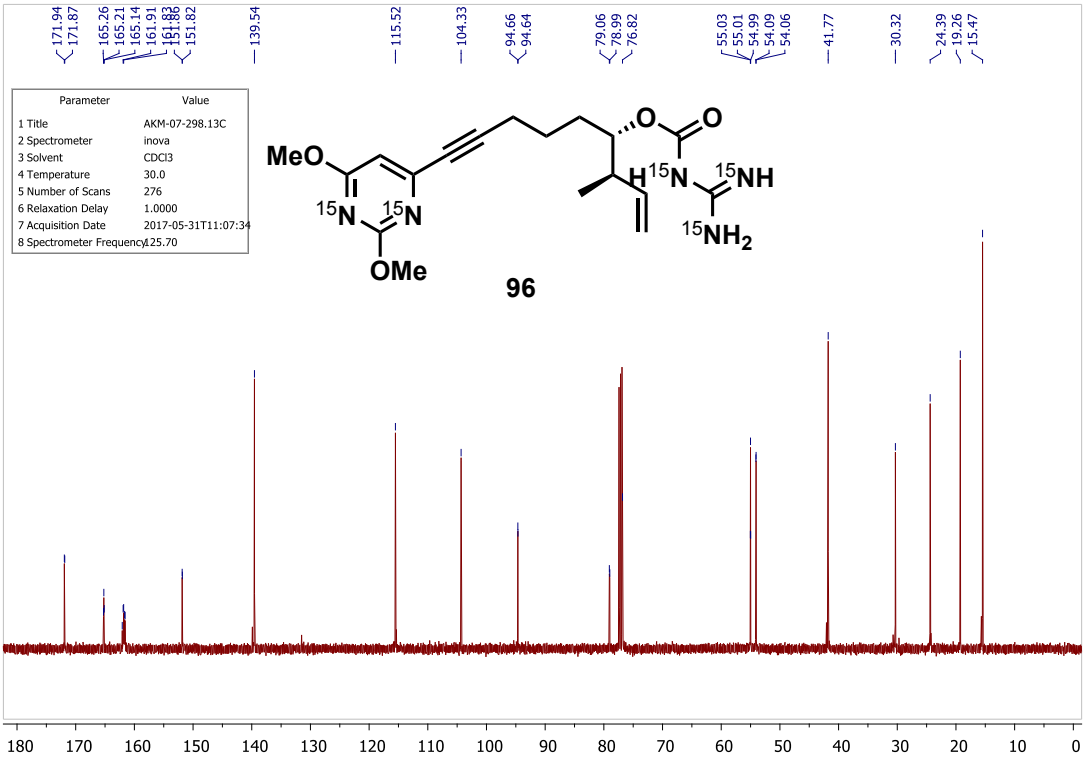

171.72
166.28
139.19
132.89
130.43
129.59
128.36
115.90
104.51
93.63
79.28
77.25
77.00
76.75
54.93
54.91
53.93
53.90
41.84
30.83
30.32
29.69
24.21
19.29
16.15
Parameter Value
1 Title jlc-3-008a-carbon1
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 22.0
5 Number of Scans 2620
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-08-19T16:07:18
8 Spectrometer Frequency 125.70
OMe
15N
15N
MeO
OBz
S2
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

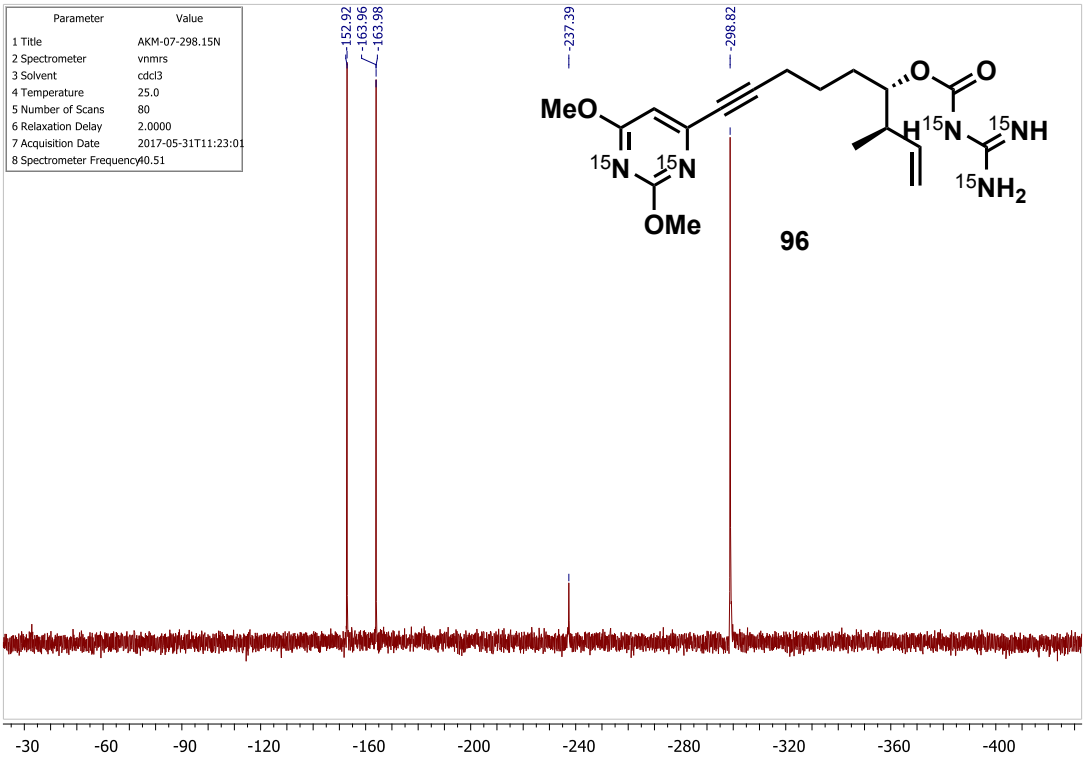

-152.47
-162.82
Parameter Value
1 Title jlc-3-008-15N
2 Spectrometer vnmrs
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 2500
6 Relaxation Delay 2.0000
7 Acquisition Date 2017-08-21T19:04:54
8 Spectrometer Frequency 40.51
OMe
15N
15N
MeO
OBz
S2
-95 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210

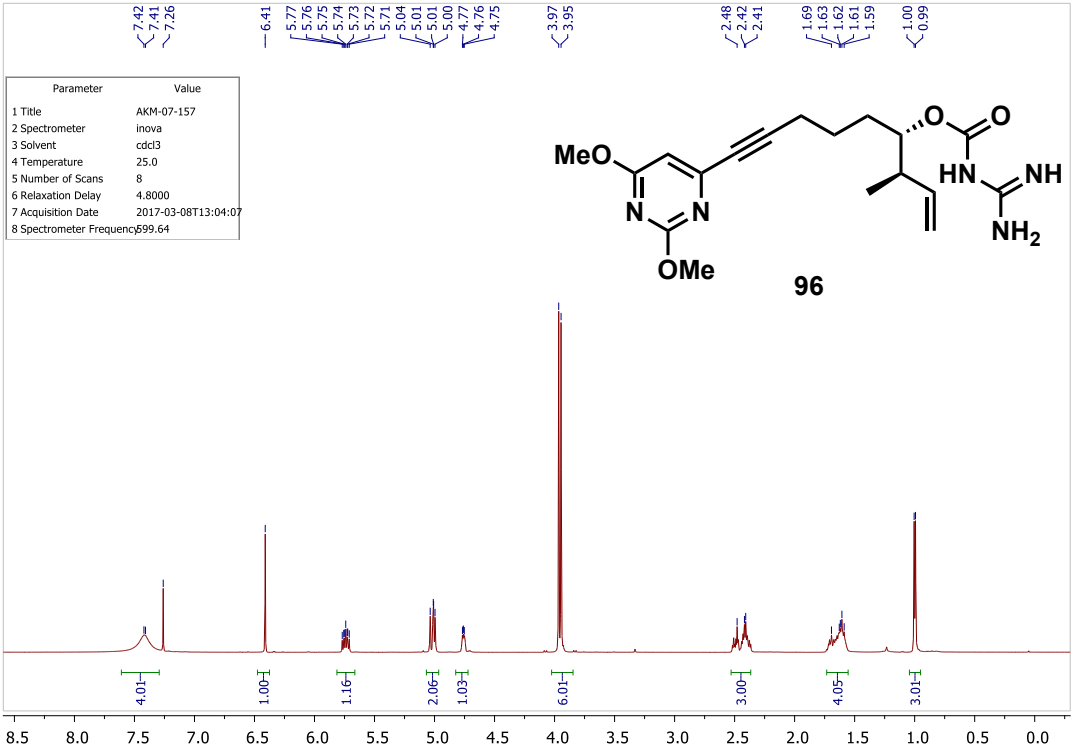

8.05
8.04
7.56
7.55
7.44
7.26
6.41
5.84
5.16
5.15
5.12
5.09
5.08
5.06
3.98
3.95
2.56
2.55
2.46
1.82
1.81
1.69
1.68
1.09
1.08
Parameter Value
1 Title jlc-3-005b-proton
2 Spectrometer inova
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 20
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-08-16T13:32:13
8 Spectrometer Frequency 599.64
OMe
N
N
MeO
OBz
S2
1.95
1.01
2.07
0.89
1.00
0.96
2.10
6.03
1.06
2.01
2.01
2.04
3.10
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

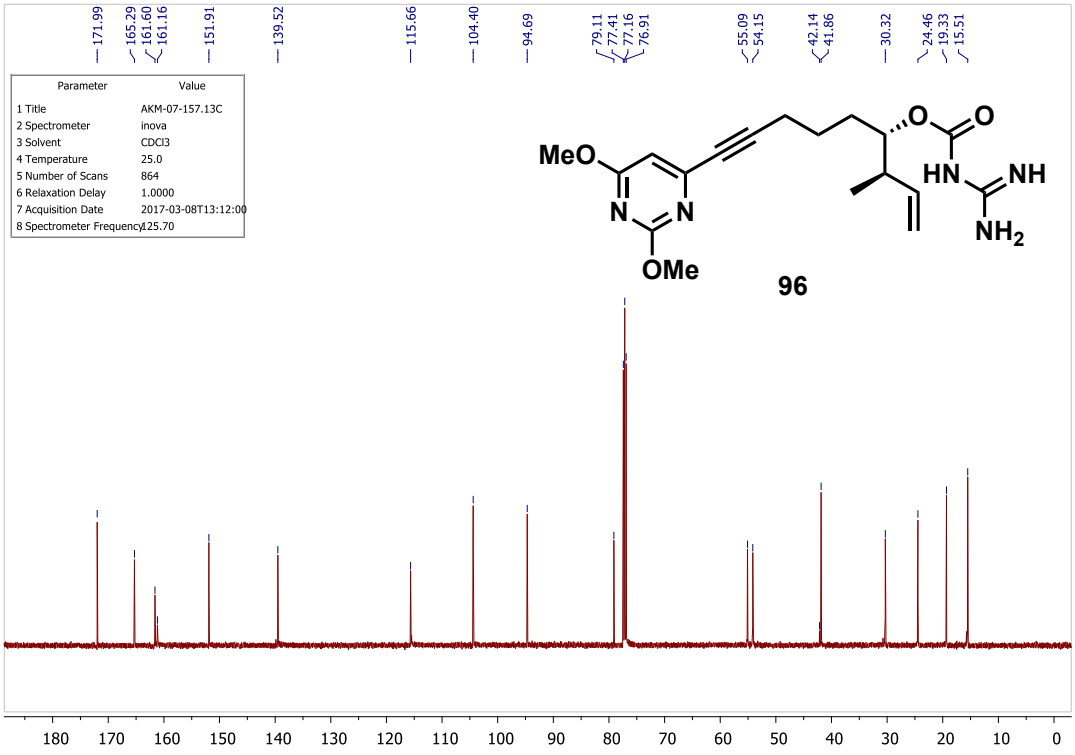

171.76
166.27
165.31
151.73
139.18
132.88
130.42
129.58
128.35
115.89
104.50
93.61
79.31
77.25
77.00
76.74
54.92
53.90
41.83
30.82
24.20
19.28
16.14
Parameter Value
1 Title jlc-3-005b-carbon
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 22.0
5 Number of Scans 1992
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-08-16T20:19:52
8 Spectrometer Frequency 125.70
OMe
N
N
MeO
OBz
S2
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10

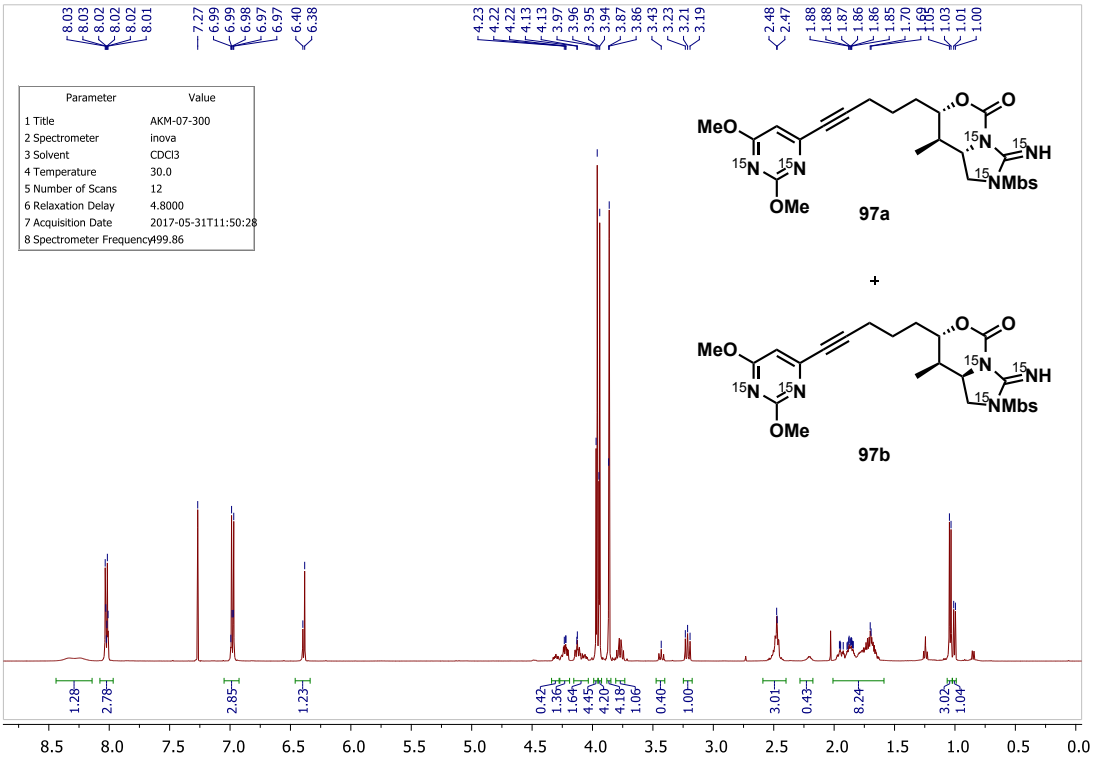

7.26
7.10
6.38
5.74
5.73
5.72
5.70
5.70
5.68
5.01
4.99
4.98
4.98
4.97
4.97
4.97
4.73
4.72
4.72
3.94
3.92
2.44
2.40
2.39
1.61
1.59
1.58
0.98
0.97
Parameter Value
1 Title AKM-07-298
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 30.0
5 Number of Scans 8
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-05-31T11:05:08
8 Spectrometer Frequency 499.86
MeO
OMe
H
OO
96
4.00
0.87
1.00
2.02
1.00
5.97
2.98
4.03
3.01
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

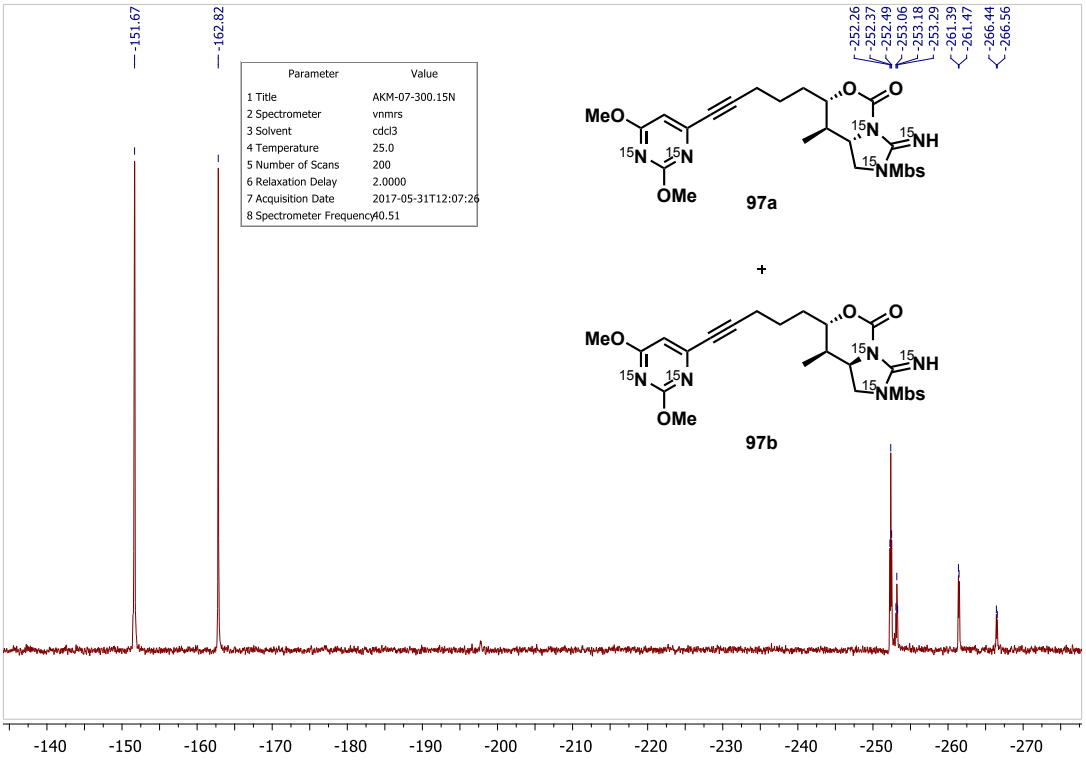

171.94
171.87
165.26
165.21
165.14
161.91
151.82
139.54
115.52
104.33
94.66
94.64
79.06
78.99
76.82
55.03
55.01
54.99
54.09
54.06
41.77
30.32
24.39
19.26
15.47
Parameter Value
1 Title AKM-07-298.13C
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 30.0
5 Number of Scans 276
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-05-31T11:07:34
8 Spectrometer Frequency 125.70
MeO
OMe
H
OO
96
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

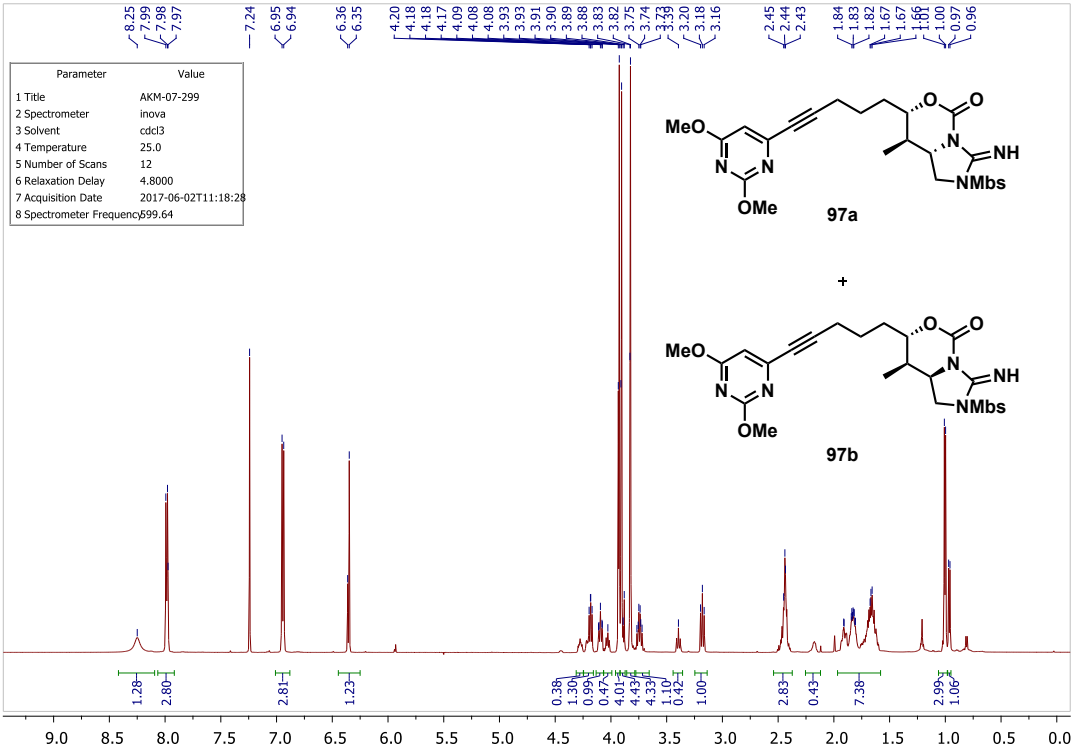

Parameter Value
1 Title AKM-07-298.15N
2 Spectrometer vnmrs
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 80
6 Relaxation Delay 2.0000
7 Acquisition Date 2017-05-31T11:23:01
8 Spectrometer Frequency 40.51
-152.92
-163.96
-163.98
-237.39
-298.82
MeO
OMe
96
-30 -60 -90 -120 -160 -200 -240 -280 -320 -360 -400

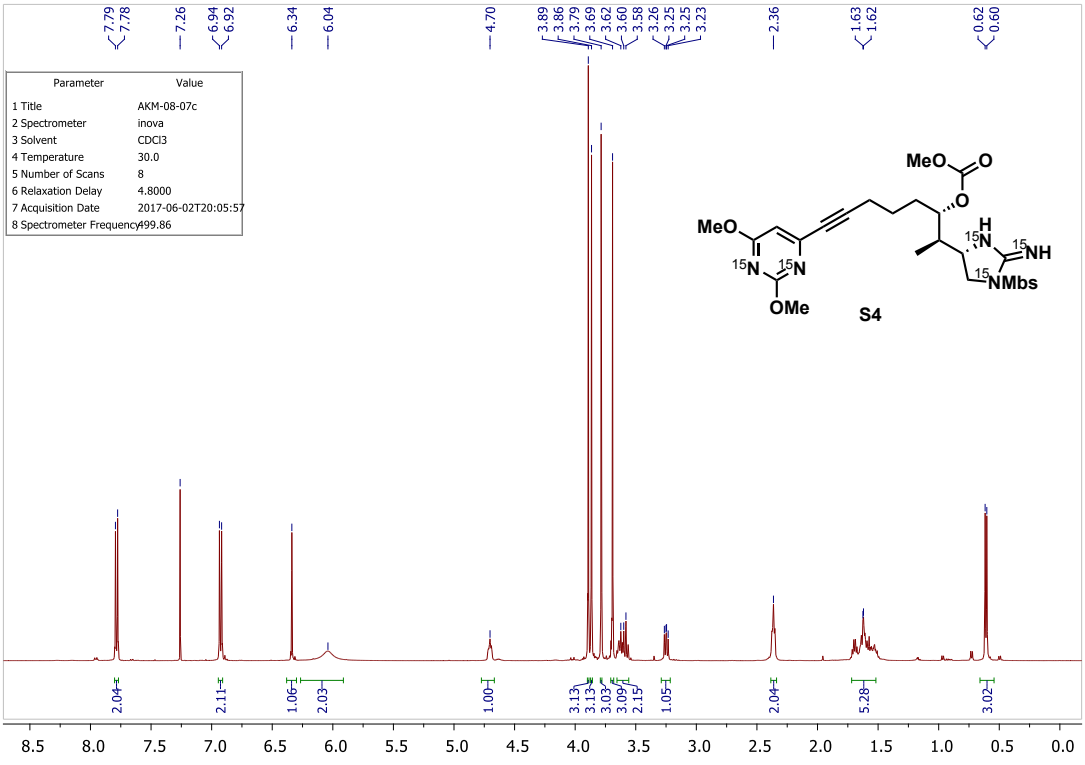

7.42 7.41 7.26
6.41
5.77 5.76 5.75 5.74 5.73 5.72 5.71
5.04 5.01 5.01 5.00
4.77 4.76 4.75
3.97 3.95
2.48 2.42 2.41
1.69 1.63 1.62 1.61 1.59
1.00 0.99
Parameter Value
1 Title AKM-07-157
2 Spectrometer inova
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 8
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-03-08T13:04:07
8 Spectrometer Frequency 599.64
MeO
OMe
96
4.01 1.00 1.16 2.06 1.03 6.01 3.00 4.05 3.01
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

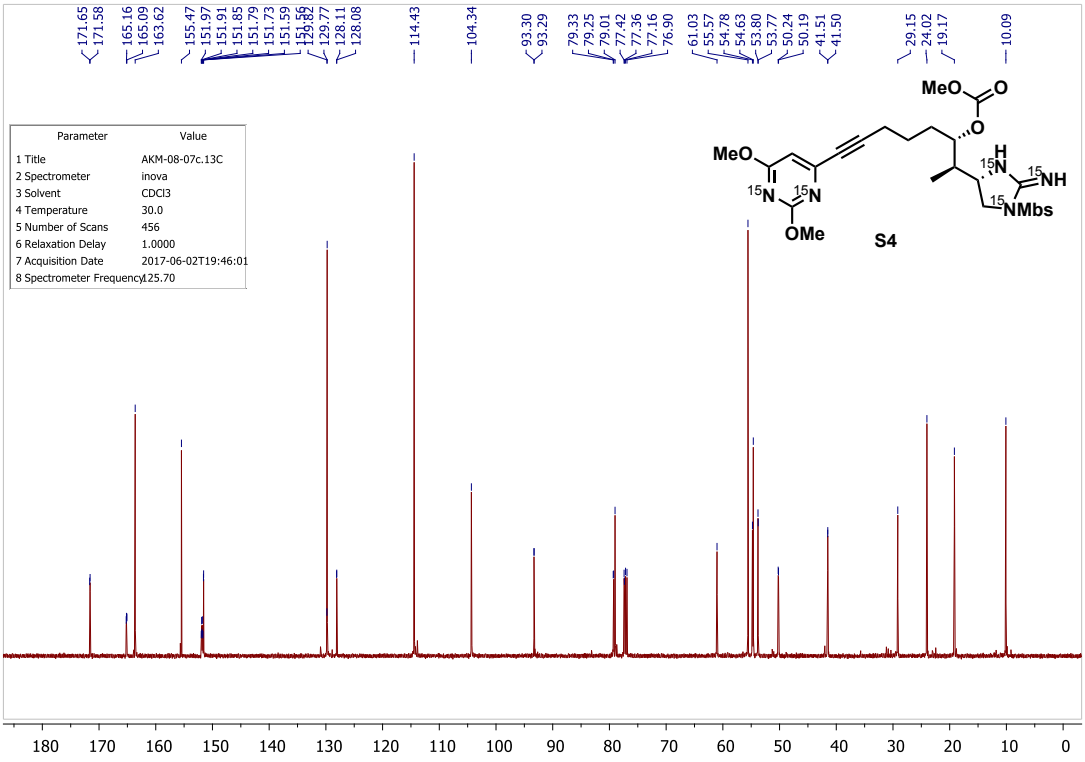

171.99
165.29
161.60
161.16
151.91
139.52
115.66
104.40
94.69
79.11
77.41
77.16
76.91
55.09
54.15
42.14
41.86
30.32
24.46
19.33
15.51
Parameter Value
1 Title AKM-07-157.13C
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 25.0
5 Number of Scans 864
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-03-08T13:12:00
8 Spectrometer Frequency 125.70
MeO
N
N
OMe
O
O
HN
NH
NH2
96
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

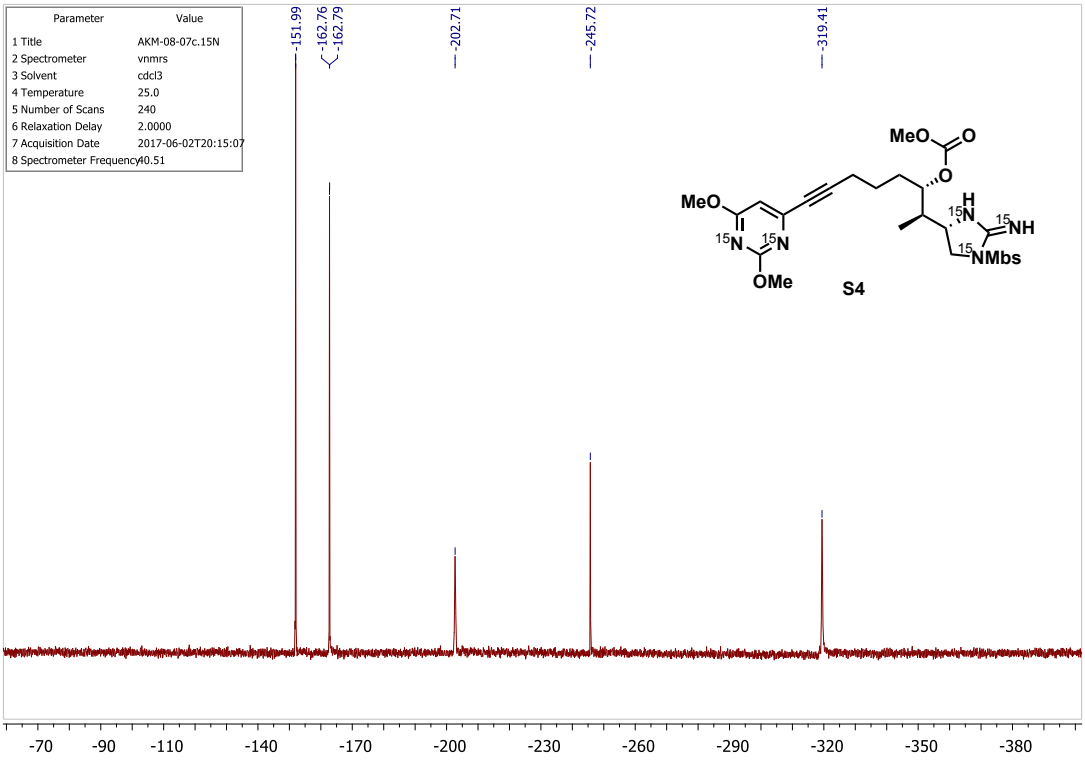

8.03
8.03
8.02
8.02
8.02
8.01
7.27
6.99
6.99
6.98
6.97
6.97
6.40
6.38
4.23
4.22
4.22
4.13
4.13
3.97
3.96
3.95
3.94
3.87
3.86
3.43
3.23
3.21
3.19
2.48
2.47
1.88
1.88
1.87
1.86
1.86
1.85
1.70
1.03
1.01
1.00
Parameter Value
1 Title AKM-07-300
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 30.0
5 Number of Scans 12
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-05-31T11:50:28
8 Spectrometer Frequency 499.86
MeO
15N
15N
OMe
O
O
15N
15NH
15NMbs
97a
+
97b
1.28
2.78
2.85
1.23
0.42
1.35
1.64
4.45
4.20
4.18
1.06
0.40
1.00
3.01
0.43
8.24
3.02
1.04
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

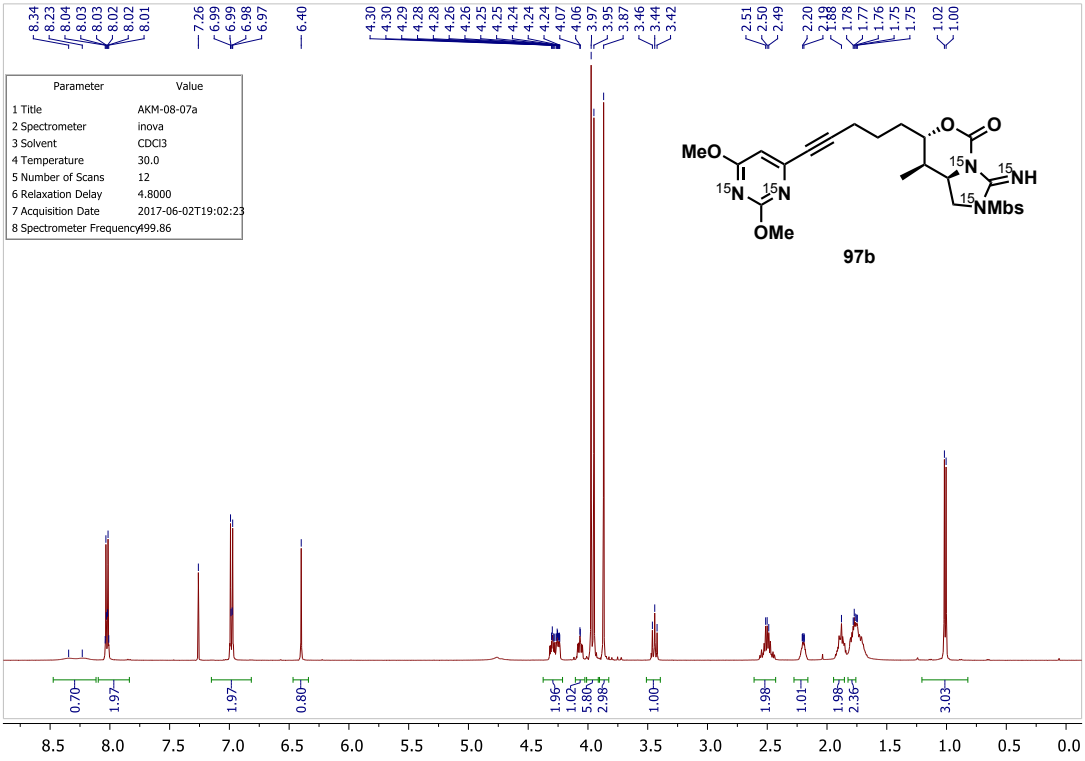

-151.67
-162.82
-252.26
-252.37
-252.49
-253.06
-253.18
-253.29
-261.39
-261.47
-266.44
-266.56
Parameter Value
1 Title AKM-07-300.15N
2 Spectrometer vnmrs
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 200
6 Relaxation Delay 2.0000
7 Acquisition Date 2017-05-31T12:07:26
8 Spectrometer Frequency 40.51
MeO
15N
15N
OMe
O
O
15N
15NH
15NMbs
97a
+
97b
-140
-150
-160
-170
-180
-190
-200
-210
-220
-230
-240
-250
-260
-270

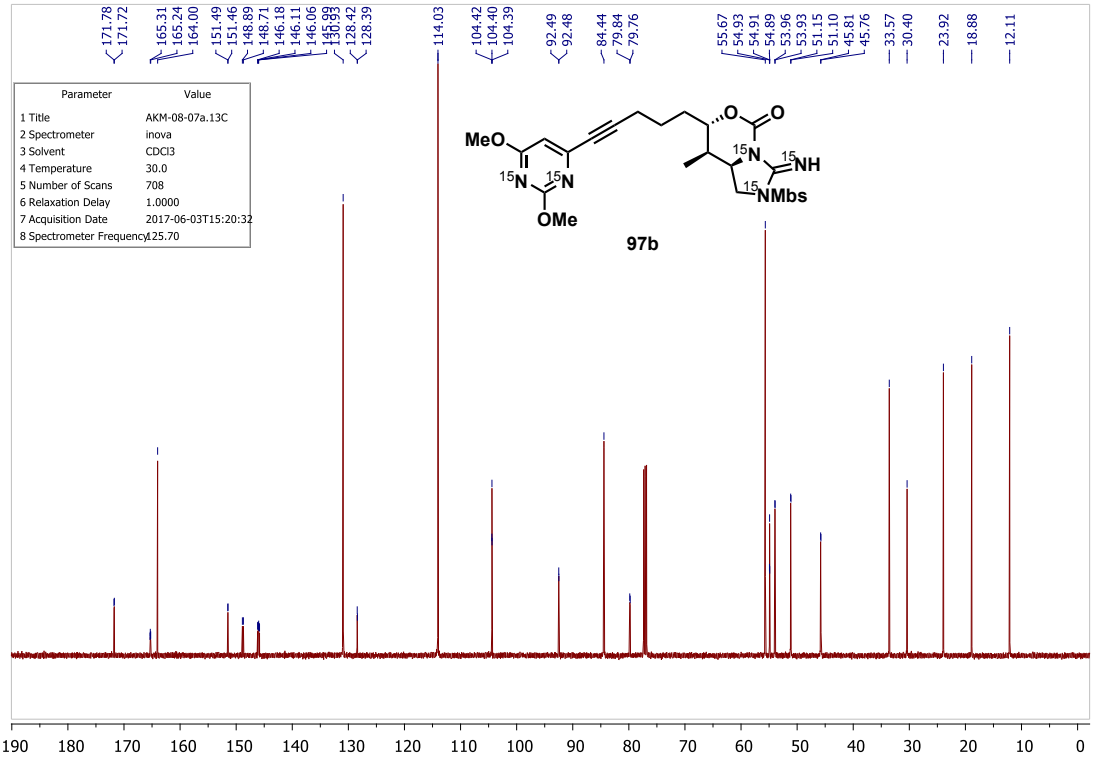

8.25
7.99
7.98
7.97
7.24
6.95
6.94
6.36
6.35
4.20
4.18
4.18
4.17
4.09
4.08
4.08
3.93
3.93
3.91
3.90
3.89
3.88
3.83
3.82
3.75
3.74
3.73
3.39
3.20
3.18
3.16
2.45
2.44
2.43
1.84
1.83
1.82
1.67
1.67
1.66
1.01
1.00
0.97
0.96
Parameter Value
1 Title AKM-07-299
2 Spectrometer inova
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 12
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-06-02T11:18:28
8 Spectrometer Frequency 599.64
MeO
N
N
OMe
O
O
N
NH
NMbs
97a
+
97b
1.28
2.80
2.81
1.23
0.38
1.30
0.99
0.47
4.01
4.43
4.33
1.10
0.42
1.00
2.83
0.43
7.38
2.99
1.06
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

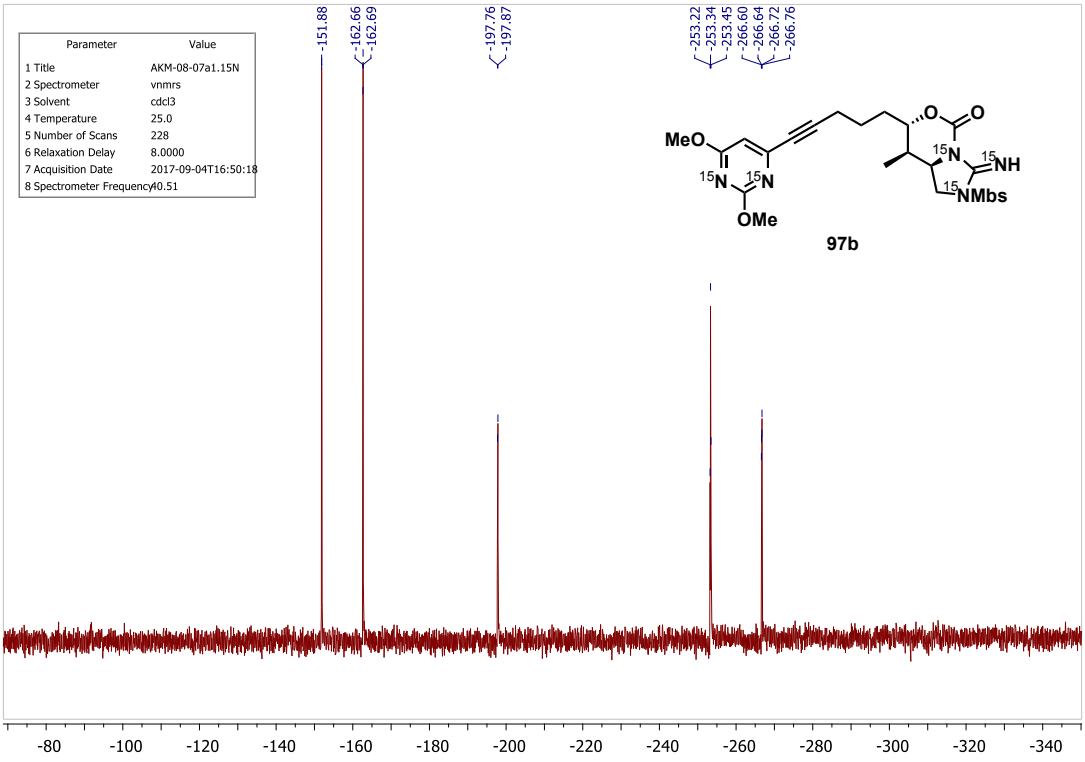

7.79
7.78
7.26
6.94
6.92
6.34
6.04
4.70
3.89
3.86
3.79
3.69
3.62
3.60
3.58
3.26
3.25
3.25
3.23
2.36
1.63
1.62
0.62
0.60
Parameter Value
1 Title AKM-08-07c
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 30.0
5 Number of Scans 8
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-06-02T20:05:57
8 Spectrometer Frequency 499.86
MeO
O
O
MeO
H
15N
15NH
15N
15N
15NMbs
OMe
S4
2.04
2.11
1.06
2.03
1.00
3.13
3.13
3.03
3.09
2.15
1.05
2.04
5.28
3.02
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

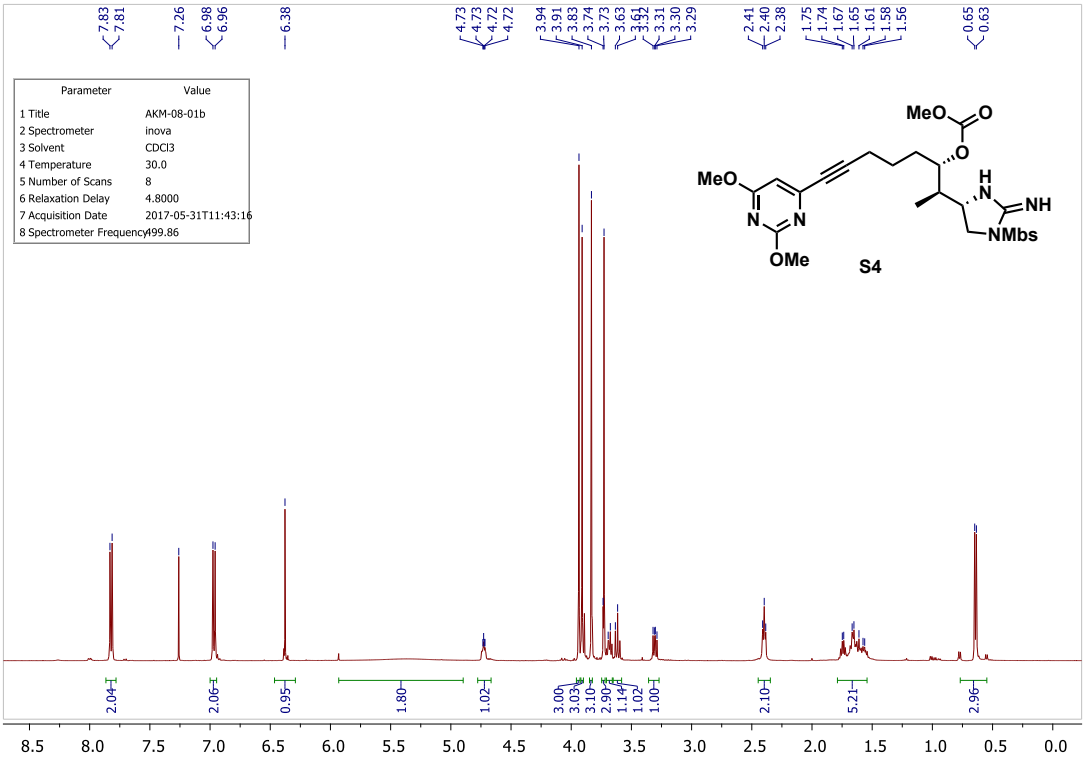

171.65
171.58
165.16
165.09
163.62
155.47
151.97
151.91
151.85
151.79
151.73
151.59
151.56
129.82
129.77
128.11
128.08
114.43
104.34
93.30
93.29
79.33
79.25
79.01
77.42
77.36
77.16
76.90
61.03
55.57
54.78
54.63
53.80
53.77
50.24
50.19
41.51
41.50
29.15
24.02
19.17
10.09
Parameter Value
1 Title AKM-08-07c.13C
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 30.0
5 Number of Scans 456
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-06-02T19:46:01
8 Spectrometer Frequency 125.70
MeO
O
O
MeO
H
15N
15NH
15N
15N
15NMbs
OMe
S4
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

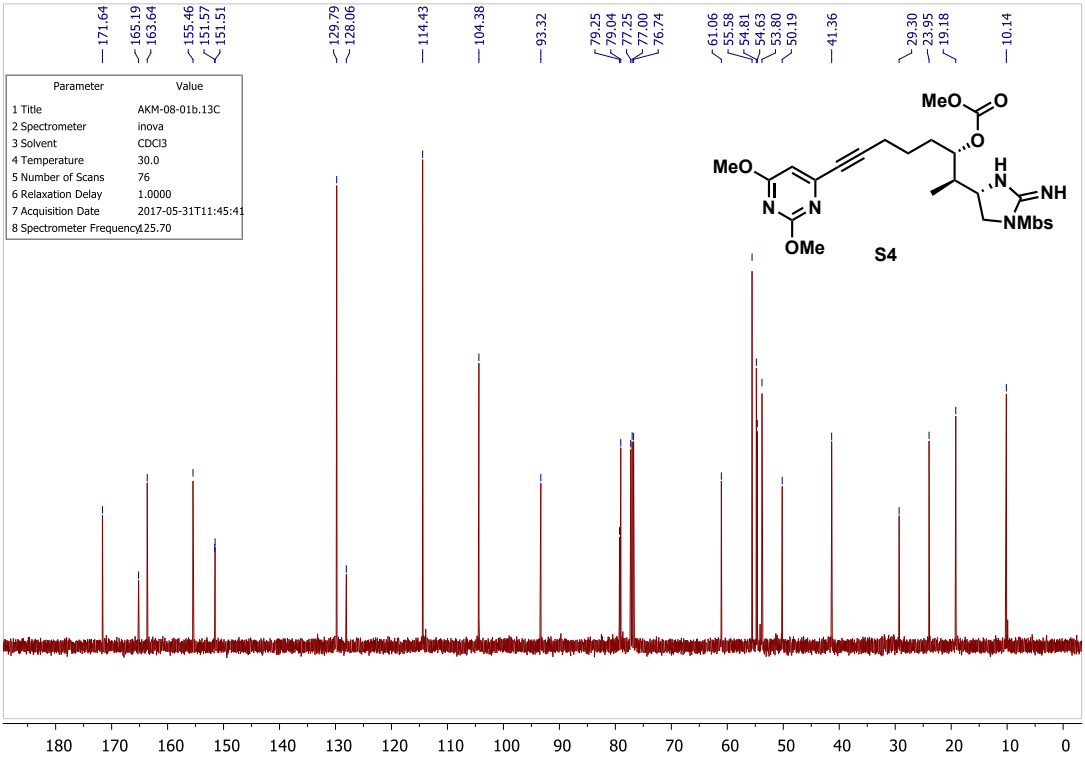

Parameter Value
1 Title AKM-08-07c.15N
2 Spectrometer vnmrs
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 240
6 Relaxation Delay 2.0000
7 Acquisition Date 2017-06-02T20:15:07
8 Spectrometer Frequency 40.51
-151.99
-162.76
-162.79
-202.71
-245.72
-319.41
MeO
OMe
S4
-70 -90 -110 -140 -170 -200 -230 -260 -290 -320 -350 -380

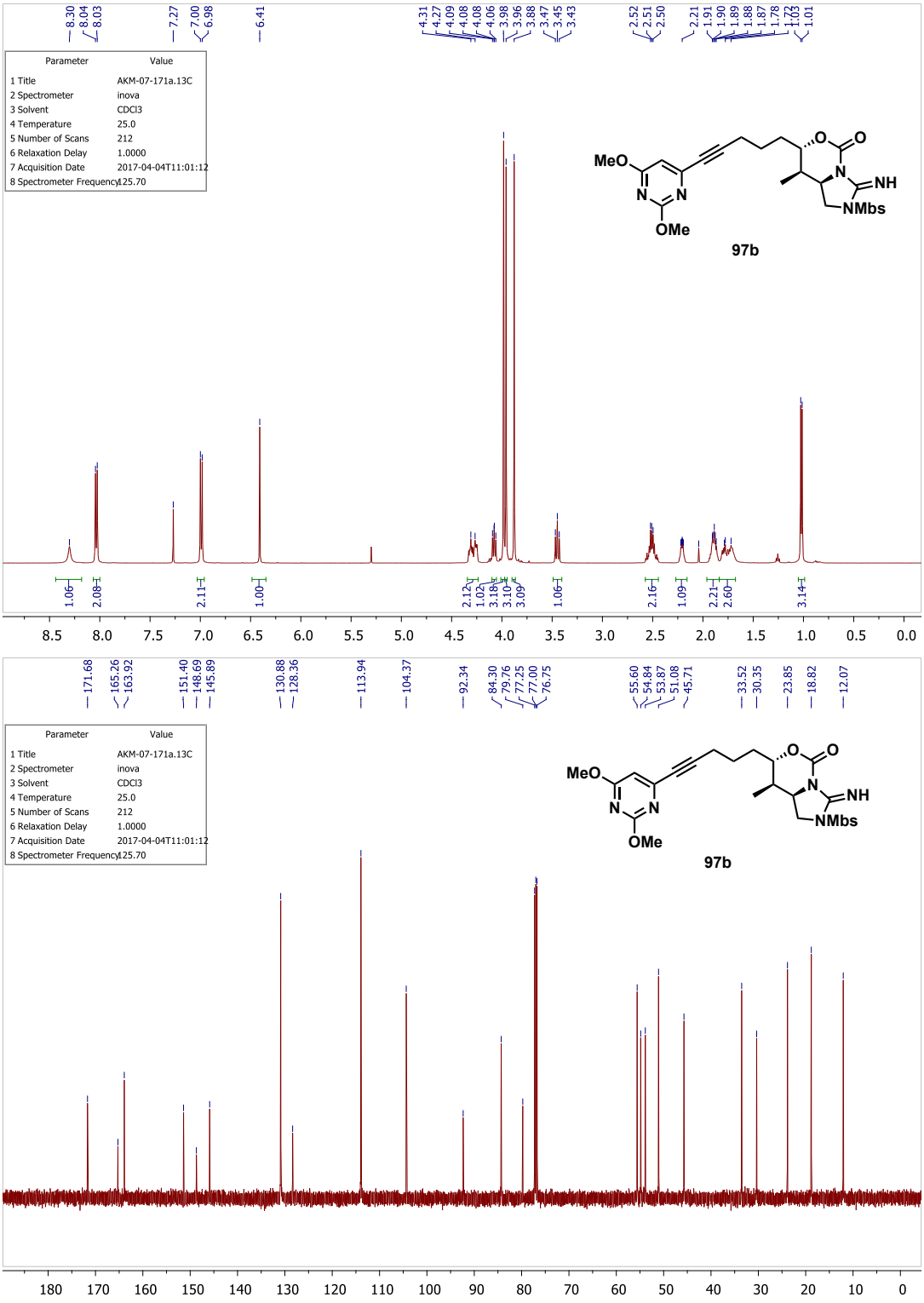

Parameter Value
1 Title AKM-08-07a
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 30.0
5 Number of Scans 12
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-06-02T19:02:23
8 Spectrometer Frequency 499.86
MeO
OMe
97b
0.70 1.97 1.97 0.80 1.96 1.02 5.80 2.98 1.00 1.98 1.01 1.98 2.36 3.03
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

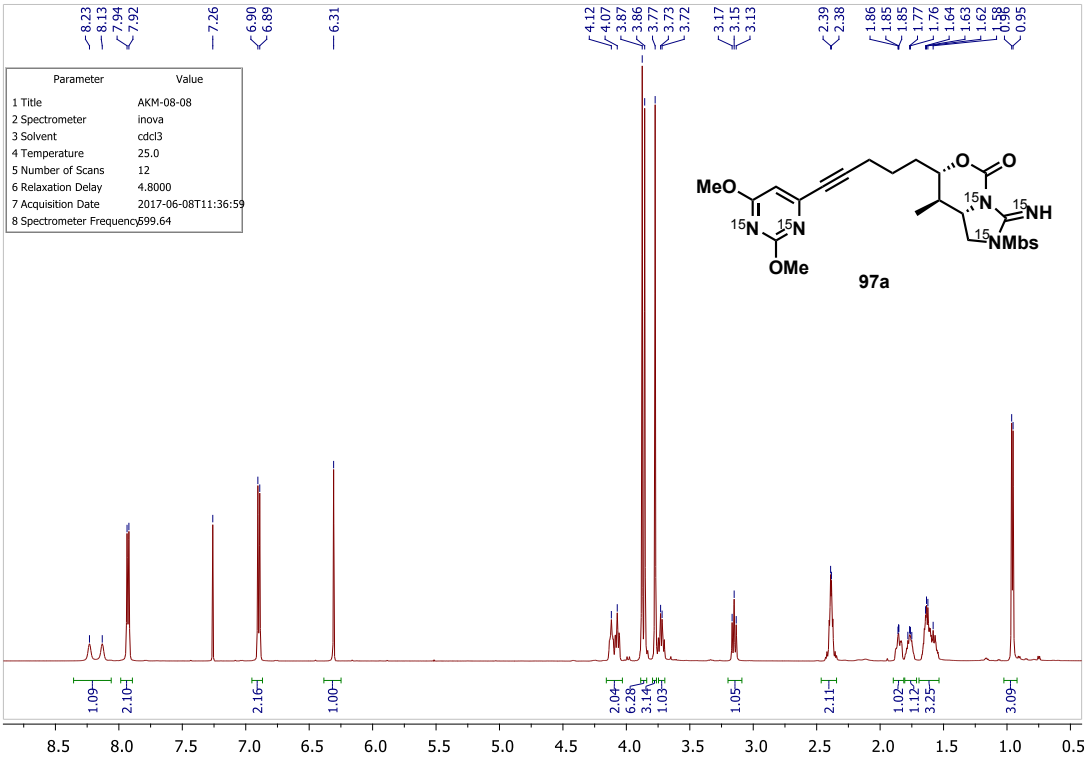

171.78
171.72
165.31
165.24
164.00
151.49
151.46
148.89
148.71
146.18
146.11
146.06
130.93
128.42
128.39
114.03
104.42
104.40
104.39
92.49
92.48
84.44
79.84
79.76
55.67
54.93
54.91
54.89
53.96
53.93
51.15
51.10
45.81
45.76
33.57
30.40
23.92
18.88
12.11
Parameter Value
1 Title AKM-08-07a.13C
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 30.0
5 Number of Scans 708
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-06-03T15:20:32
8 Spectrometer Frequency 125.70
MeO
OMe
NMbs
NH
97b
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

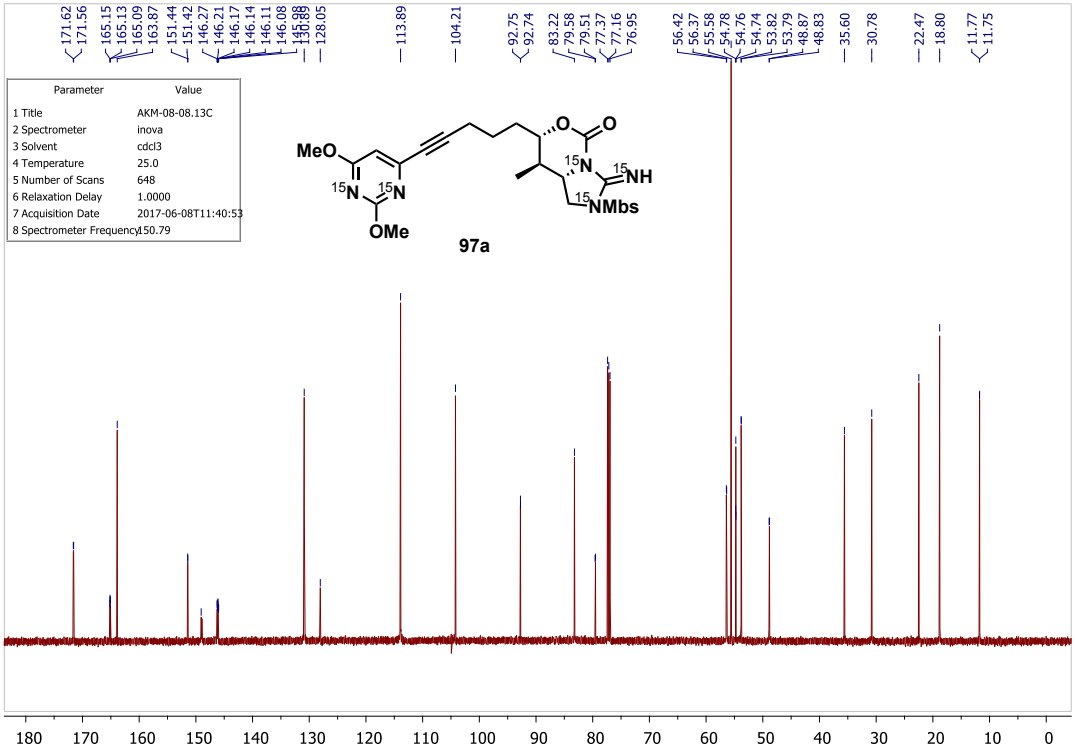

-151.88
-162.66
-162.69
-197.76
-197.87
-253.22
-253.34
-253.45
-266.60
-266.64
-266.72
-266.76
Parameter Value
1 Title AKM-08-07a1.15N
2 Spectrometer vnmrs
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 228
6 Relaxation Delay 8.0000
7 Acquisition Date 2017-09-04T16:50:18
8 Spectrometer Frequency 40.51
MeO
OMe
NMbs
NH
97b
-80 -100 -120 -140 -160 -180 -200 -220 -240 -260 -280 -300 -320 -340

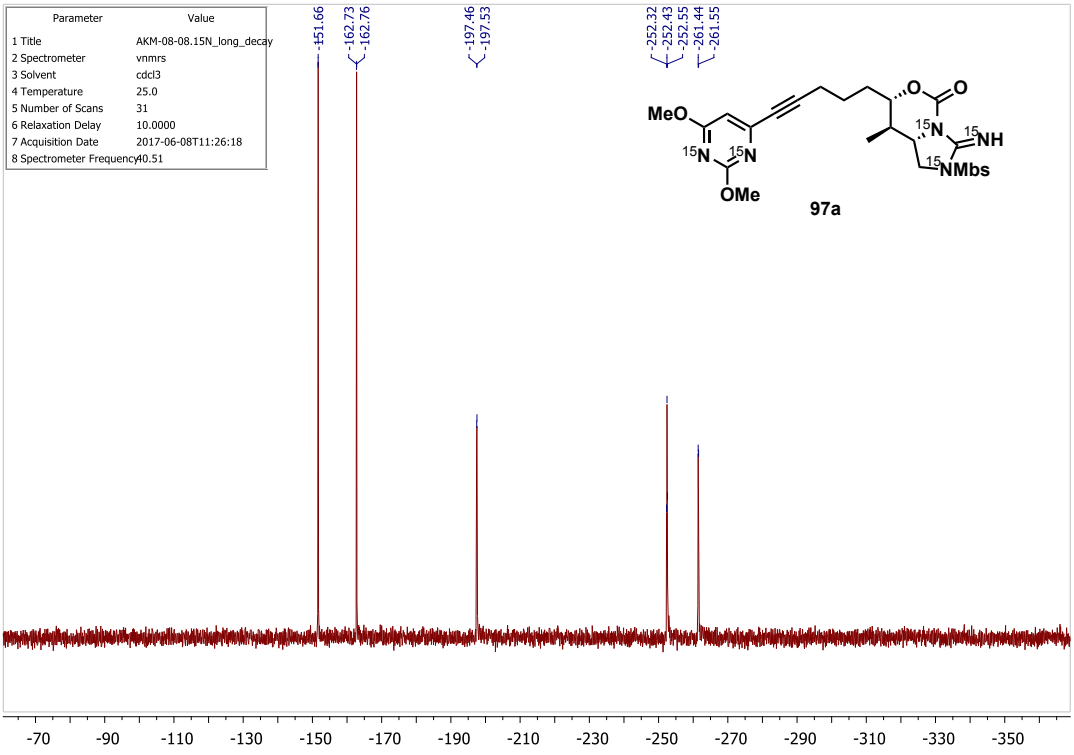

7.83
7.81
7.26
6.98
6.96
6.38
4.73
4.73
4.72
4.72
3.94
3.91
3.83
3.74
3.73
3.63
3.61
3.32
3.31
3.30
3.29
2.41
2.40
2.38
1.75
1.74
1.67
1.65
1.61
1.58
1.56
0.65
0.63
Parameter Value
1 Title AKM-08-01b
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 30.0
5 Number of Scans 8
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-05-31T11:43:16
8 Spectrometer Frequency 499.86
MeO
O
O
MeO
H
N
NH
N
N
NMbs
OMe
S4
2.04
2.06
0.95
1.80
1.02
3.00
3.03
3.10
2.90
1.14
1.02
1.00
2.10
5.21
2.96
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

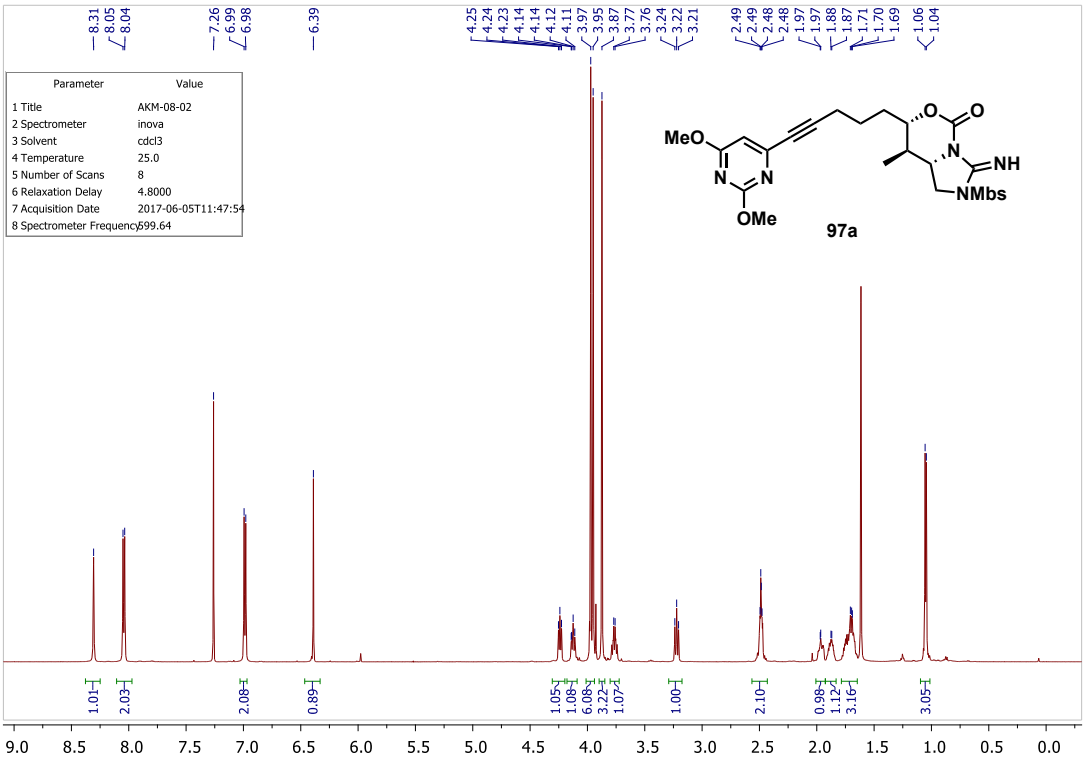

171.64
165.19
163.64
155.46
151.57
151.51
129.79
128.06
114.43
104.38
93.32
79.25
79.04
77.25
77.00
76.74
61.06
55.58
54.81
54.63
53.80
50.19
41.36
29.30
23.95
19.18
10.14
Parameter Value
1 Title AKM-08-01b.13C
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 30.0
5 Number of Scans 76
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-05-31T11:45:41
8 Spectrometer Frequency 125.70
MeO
O
O
MeO
H
N
NH
N
N
NMbs
OMe
S4
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

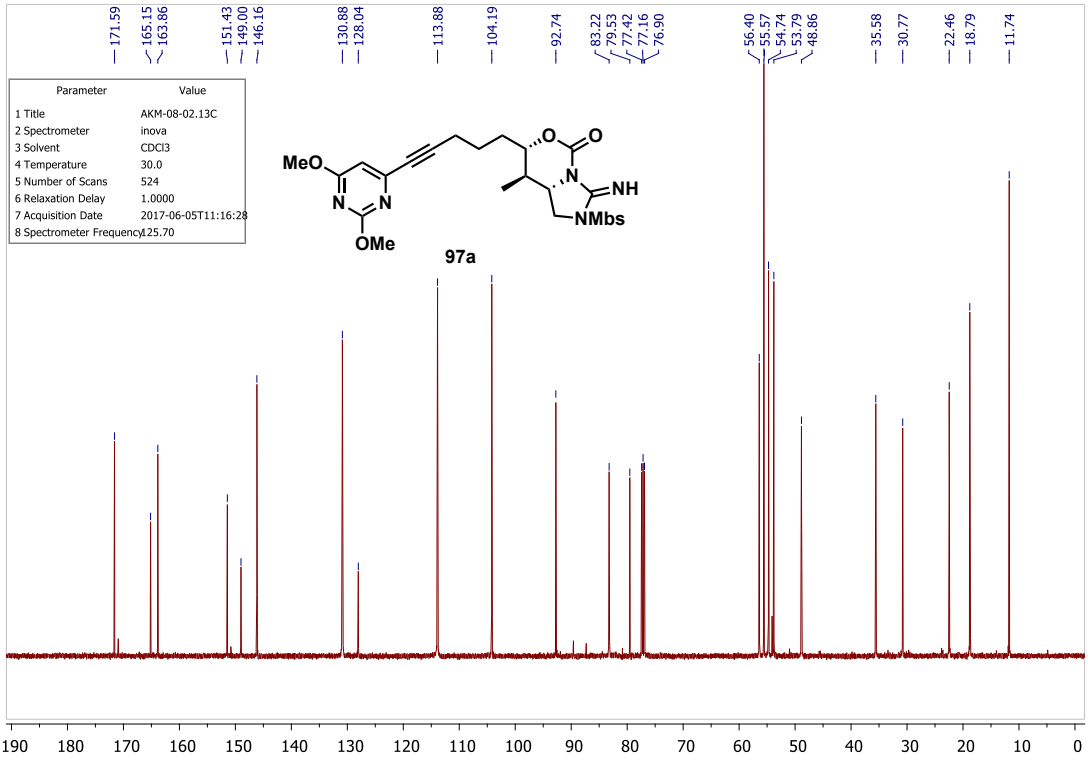

Parameter Value
1 Title AKM-07-171a.13C
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 25.0
5 Number of Scans 212
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-04-04T11:01:12
8 Spectrometer Frequency 125.70
MeO
OMe
NH
NMbs
97b

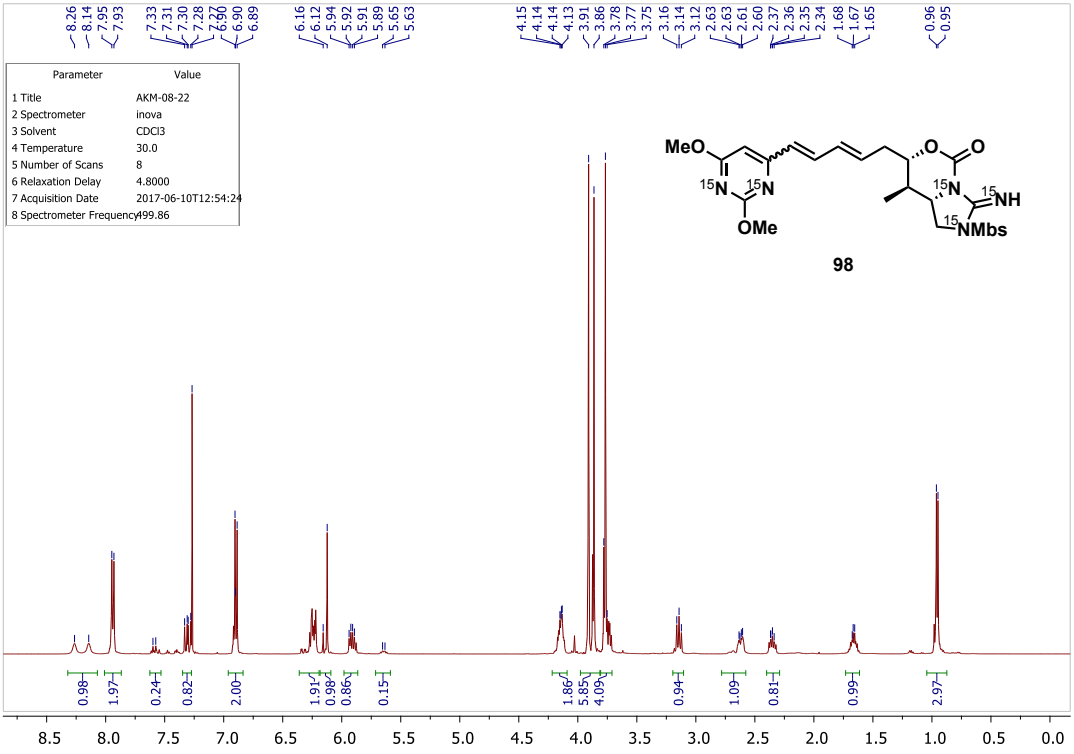

Parameter Value
1 Title AKM-08-08
2 Spectrometer inova
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 12
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-06-08T11:36:59
8 Spectrometer Frequency 599.64
MeO
OMe
NMbs
NH
97a

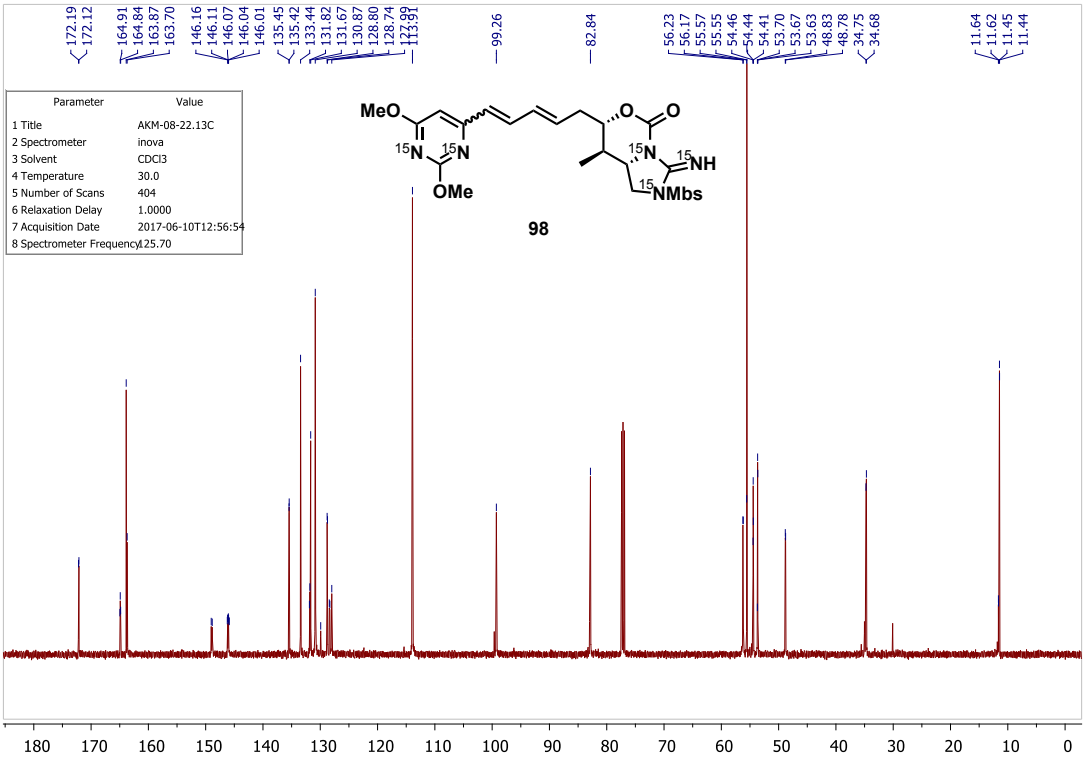

Parameter Value
1 Title AKM-08-08.13C
2 Spectrometer inova
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 648
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-06-08T11:40:53
8 Spectrometer Frequency 150.79
MeO
OMe
NMbs
NH
97a

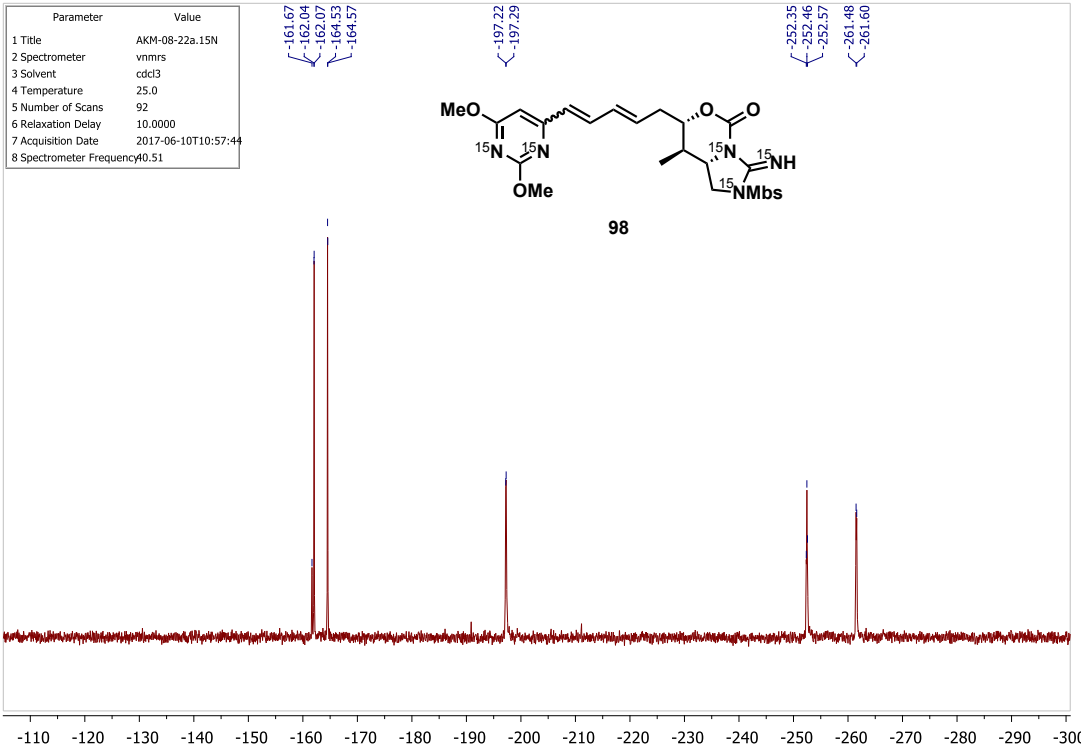

Parameter Value
1 Title AKM-08-08.15N_long_decay
2 Spectrometer vnmrs
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 31
6 Relaxation Delay 10.0000
7 Acquisition Date 2017-06-08T11:26:18
8 Spectrometer Frequency 40.51
-151.66
-162.73
-162.76
-197.46
-197.53
-252.32
-252.43
-252.55
-261.44
-261.55
MeO
OMe
NMbs
NH
97a
-70 -90 -110 -130 -150 -170 -190 -210 -230 -250 -270 -290 -310 -330 -350

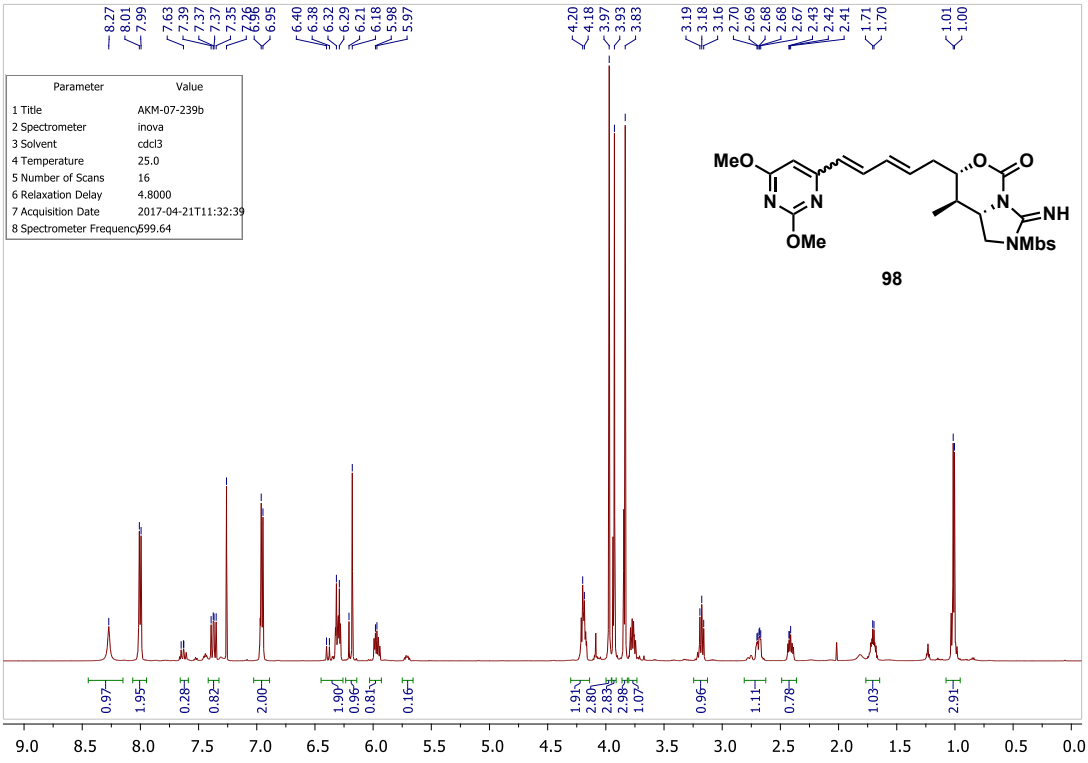

Parameter Value
1 Title AKM-08-02
2 Spectrometer inova
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 8
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-06-05T11:47:54
8 Spectrometer Frequency 599.64
8.31
8.05
8.04
7.26
6.99
6.98
6.39
4.25
4.24
4.23
4.14
4.14
4.12
4.11
3.97
3.95
3.87
3.77
3.76
3.24
3.22
3.21
2.49
2.49
2.48
2.48
1.97
1.97
1.88
1.87
1.71
1.70
1.69
1.06
1.04
MeO
OMe
NMbs
NH
97a
1.01
2.03
2.08
0.89
1.05
1.08
6.08
3.22
1.07
1.00
2.10
0.98
1.12
3.16
3.05
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

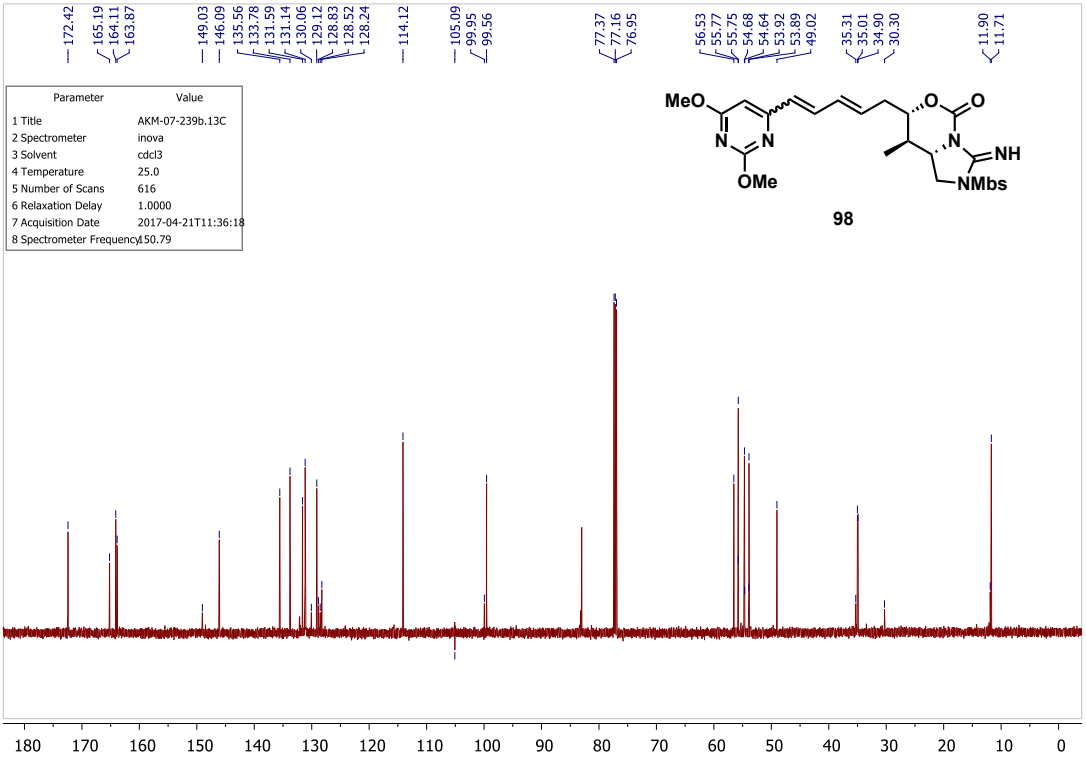

171.59
165.15
163.86
151.43
149.00
146.16
130.88
128.04
113.88
104.19
92.74
83.22
79.53
77.42
77.16
76.90
56.40
55.57
54.74
53.79
48.86
35.58
30.77
22.46
18.79
11.74
Parameter Value
1 Title AKM-08-02.13C
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 30.0
5 Number of Scans 524
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-06-05T11:16:28
8 Spectrometer Frequency 125.70
MeO
N
N
OMe
O
O
N
NH
NMbs
97a
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

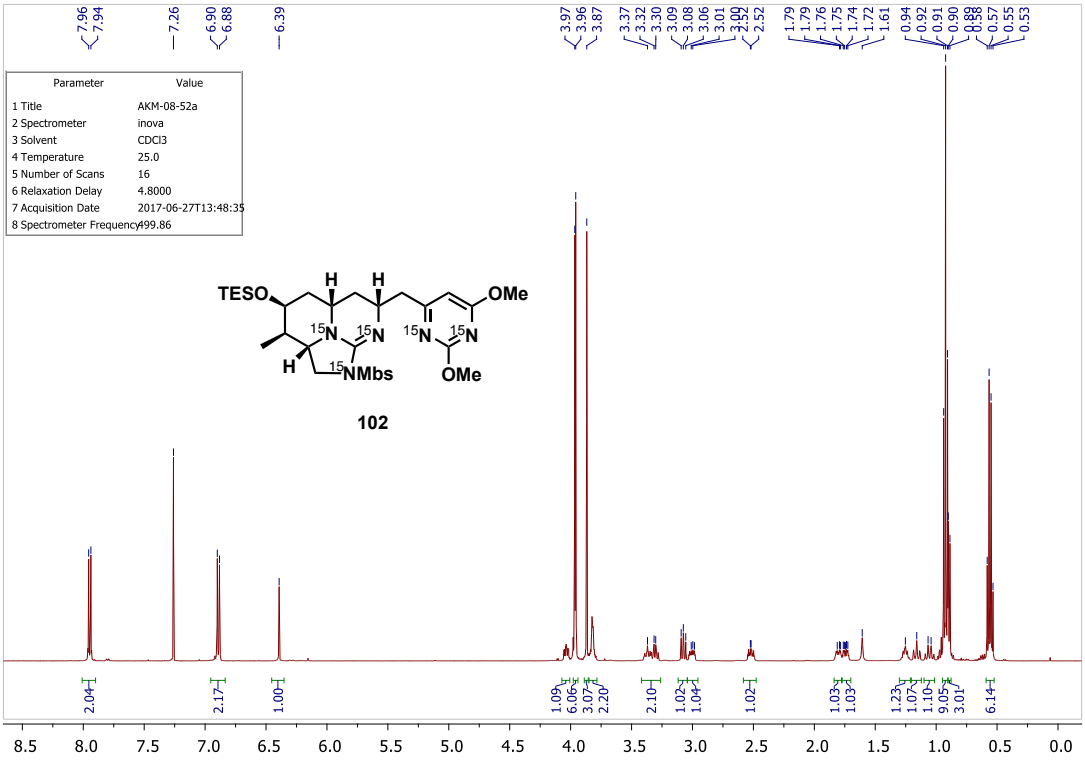

8.26
8.14
7.95
7.93
7.33
7.31
7.30
7.28
7.27
6.90
6.90
6.89
6.16
6.12
5.94
5.92
5.91
5.89
5.65
5.63
4.15
4.14
4.14
4.13
3.91
3.86
3.78
3.77
3.75
3.16
3.14
3.12
2.63
2.63
2.61
2.60
2.37
2.36
2.35
2.34
1.68
1.67
1.65
0.96
0.95
Parameter Value
1 Title AKM-08-22
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 30.0
5 Number of Scans 8
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-06-10T12:54:24
8 Spectrometer Frequency 499.86
MeO
15N
15N
OMe
O
O
15N
15
NH
15
NMbs
98
0.98
1.97
0.24
0.82
2.00
1.91
0.98
0.86
0.15
1.86
5.85
4.09
0.94
1.09
0.81
0.99
2.97
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

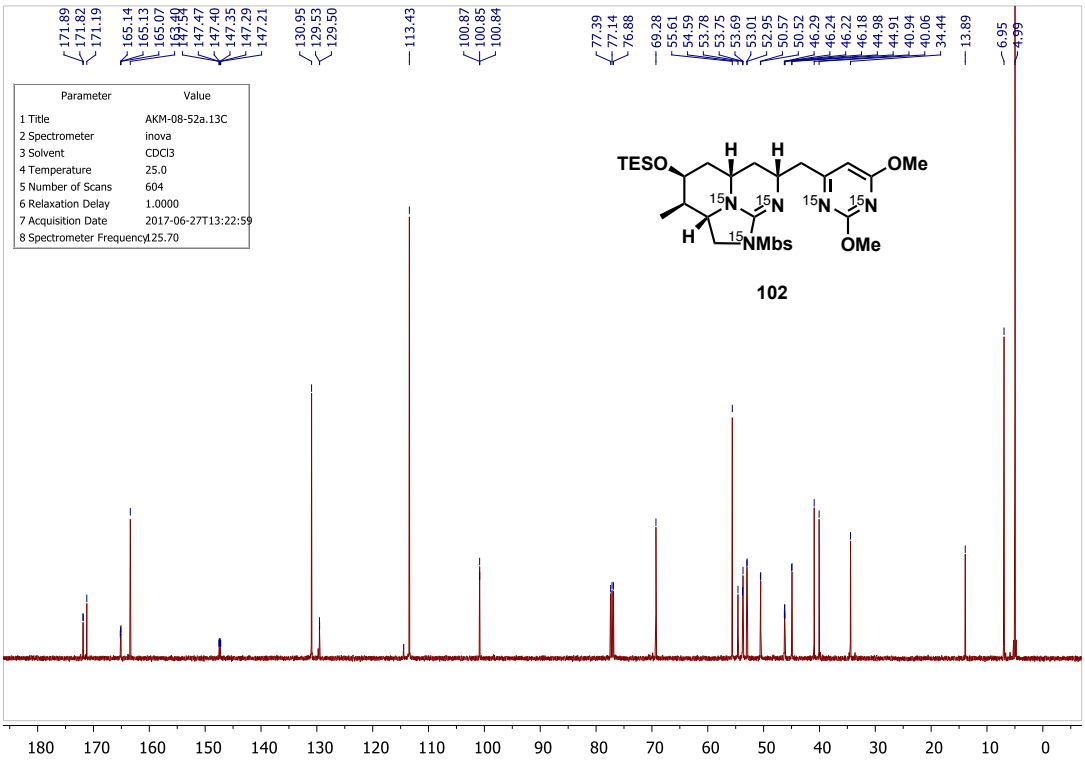

172.19
172.12
164.91
164.84
163.87
163.70
146.16
146.11
146.07
146.04
146.01
135.45
135.42
133.44
131.82
131.67
130.87
128.80
128.74
123.99
115.91
99.26
82.84
56.23
56.17
55.57
55.55
54.46
54.44
54.41
53.70
53.67
53.63
48.83
48.78
34.75
34.68
11.64
11.62
11.45
11.44
Parameter Value
1 Title AKM-08-22.13C
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 30.0
5 Number of Scans 404
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-06-10T12:56:54
8 Spectrometer Frequency 125.70
MeO
OMe
NMbs
NH
98
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

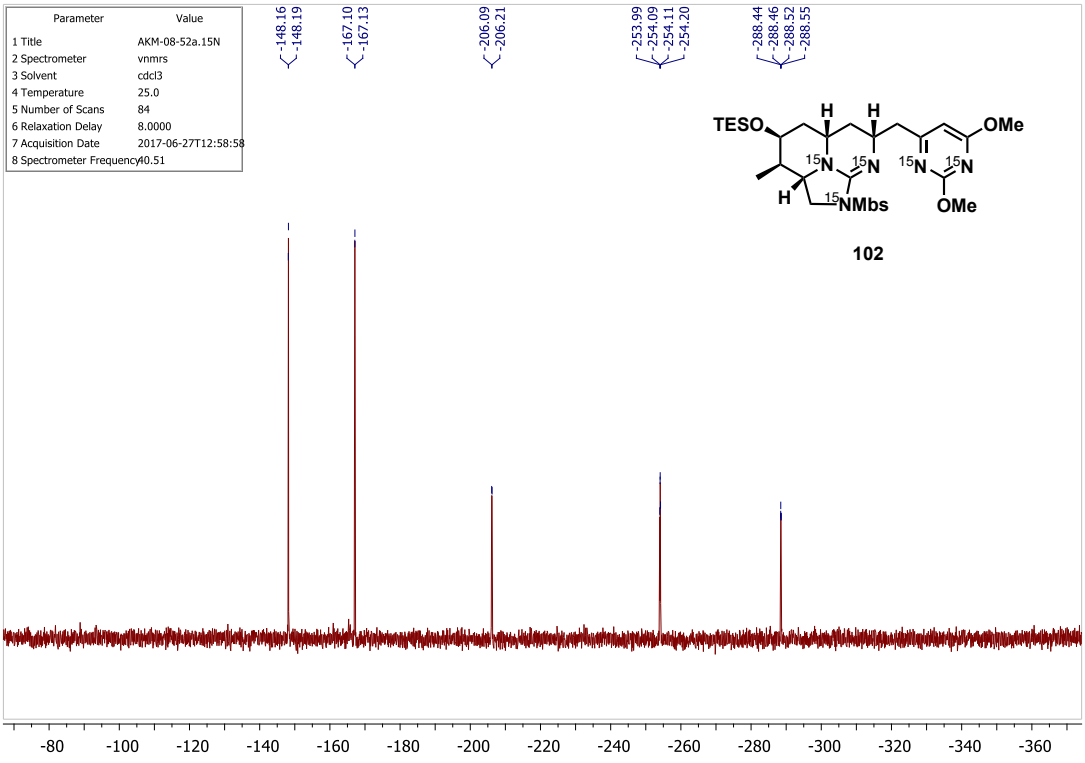

-161.67
-162.04
-162.07
-164.53
-164.57
-197.22
-197.29
-252.35
-252.46
-252.57
-261.48
-261.60
Parameter Value
1 Title AKM-08-22a.15N
2 Spectrometer vnmrs
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 92
6 Relaxation Delay 10.0000
7 Acquisition Date 2017-06-10T10:57:44
8 Spectrometer Frequency 40.51
MeO
OMe
NMbs
NH
98
-110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210 -220 -230 -240 -250 -260 -270 -280 -290 -300

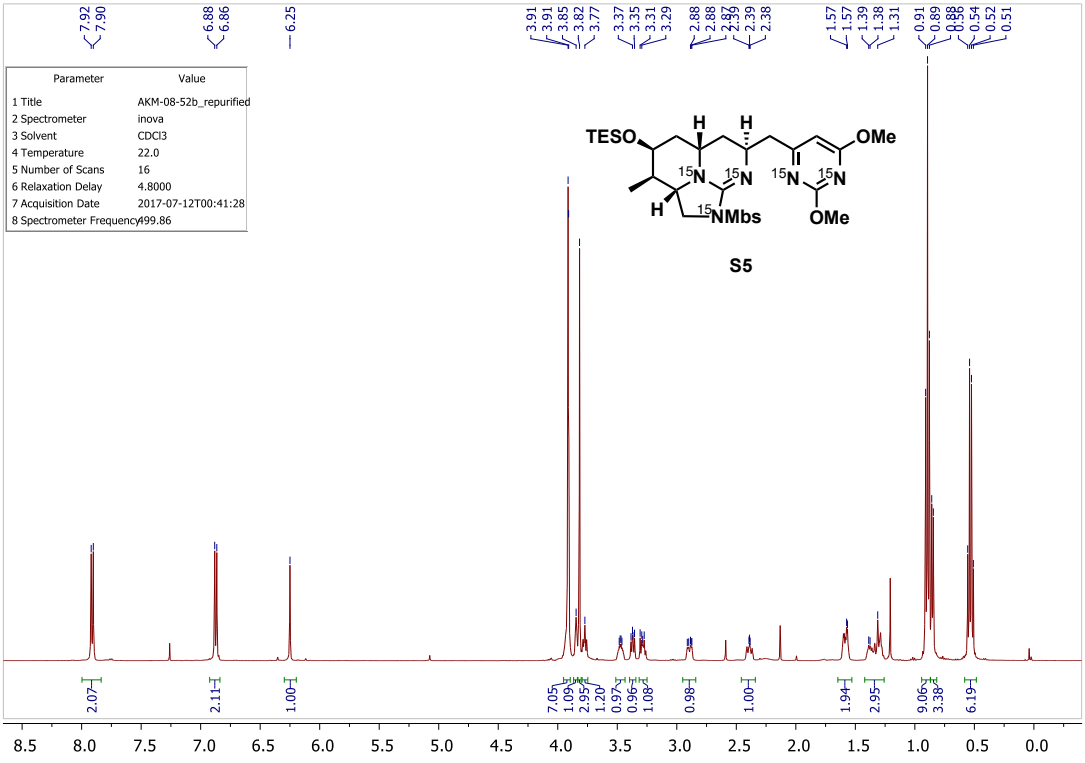

8.27
8.01
7.99
7.63
7.39
7.37
7.37
7.35
7.26
6.96
6.95
6.40
6.38
6.32
6.29
6.21
6.18
5.98
5.97
4.20
4.18
3.97
3.93
3.83
3.19
3.18
3.16
2.70
2.69
2.68
2.68
2.67
2.43
2.42
2.41
1.71
1.70
1.01
1.00
Parameter Value
1 Title AKM-07-239b
2 Spectrometer inova
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 16
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-04-21T11:32:39
8 Spectrometer Frequency 599.64
MeO
N
N
OMe
O
O
N
NH
NMbs
98
0.97
1.95
0.28
0.82
2.00
1.90
0.96
0.81
0.16
1.91
2.80
2.83
2.98
1.07
0.96
1.11
0.78
1.03
2.91
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

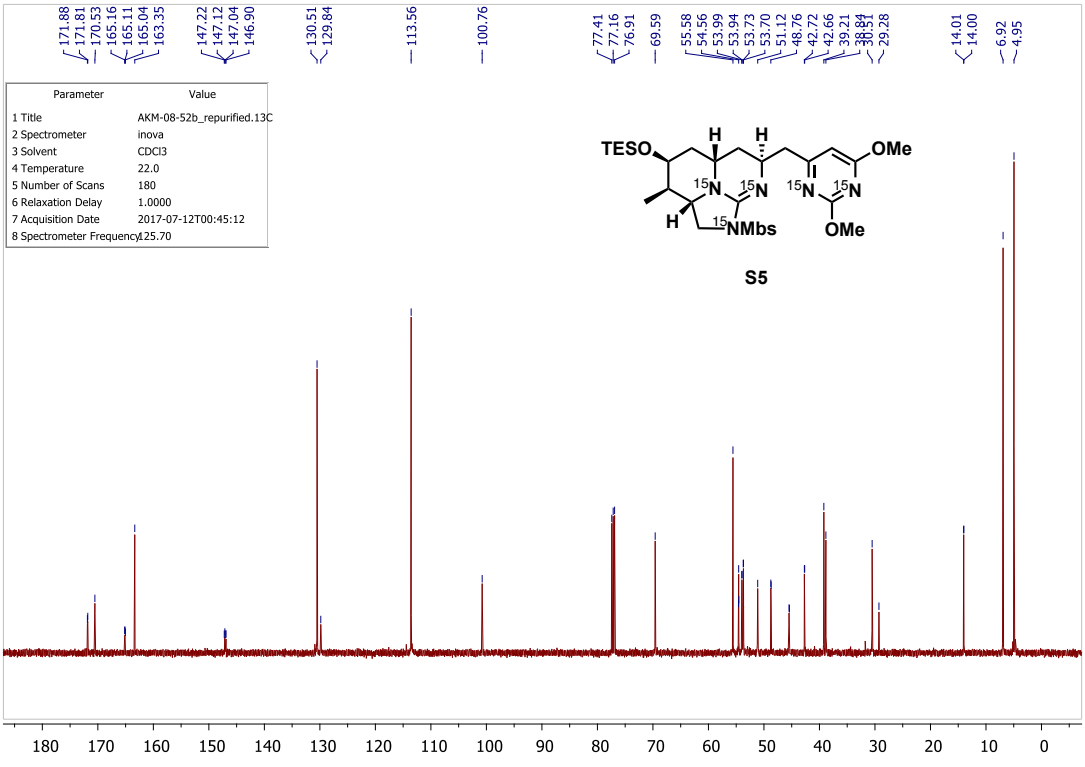

172.42
165.19
164.11
163.87
149.03
146.09
135.56
133.78
131.59
131.14
130.06
129.12
128.83
128.52
128.24
114.12
105.09
99.95
99.56
77.37
77.16
76.95
56.53
55.77
55.75
54.68
54.64
53.92
53.89
49.02
35.31
35.01
34.90
30.30
11.90
11.71
Parameter Value
1 Title AKM-07-239b.13C
2 Spectrometer inova
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 616
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-04-21T11:36:18
8 Spectrometer Frequency 150.79
MeO
N
N
OMe
O
O
N
NH
NMbs
98
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

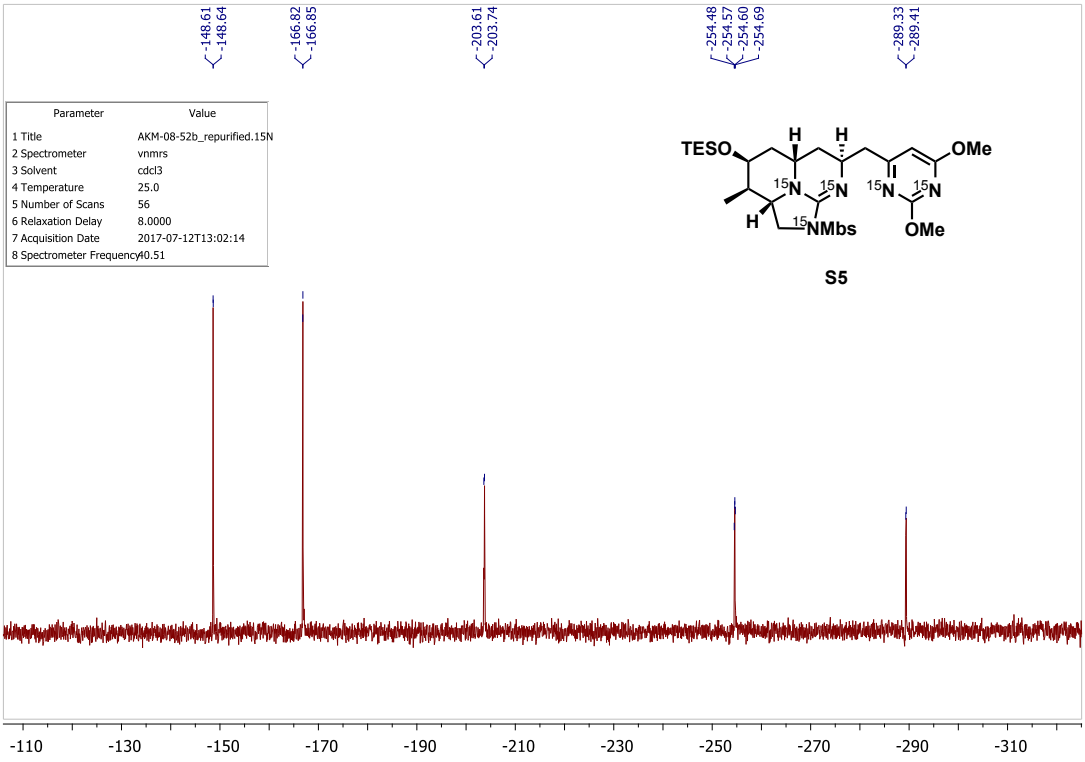

Parameter Value
1 Title AKM-08-52a
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 25.0
5 Number of Scans 16
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-06-27T13:48:35
8 Spectrometer Frequency 499.86
TESO
OMe
NMbs
OMe
102

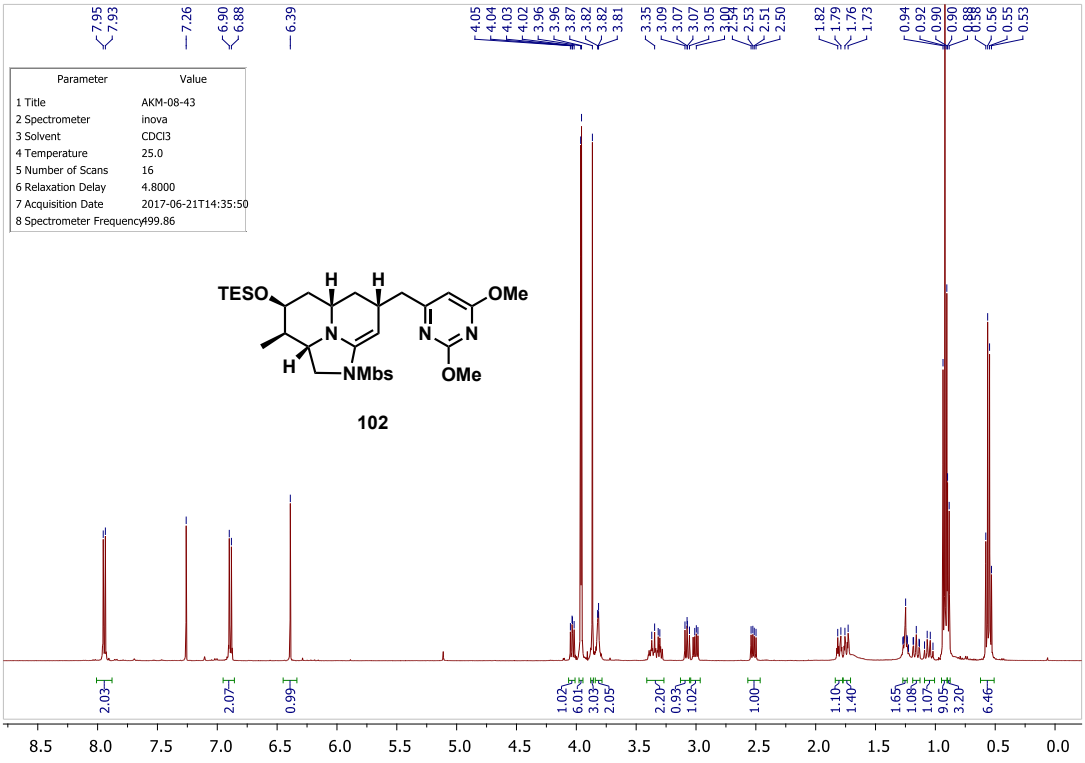

Parameter Value
1 Title AKM-08-52a.13C
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 25.0
5 Number of Scans 604
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-06-27T13:22:59
8 Spectrometer Frequency 125.70
TESO
OMe
NMbs
OMe
102

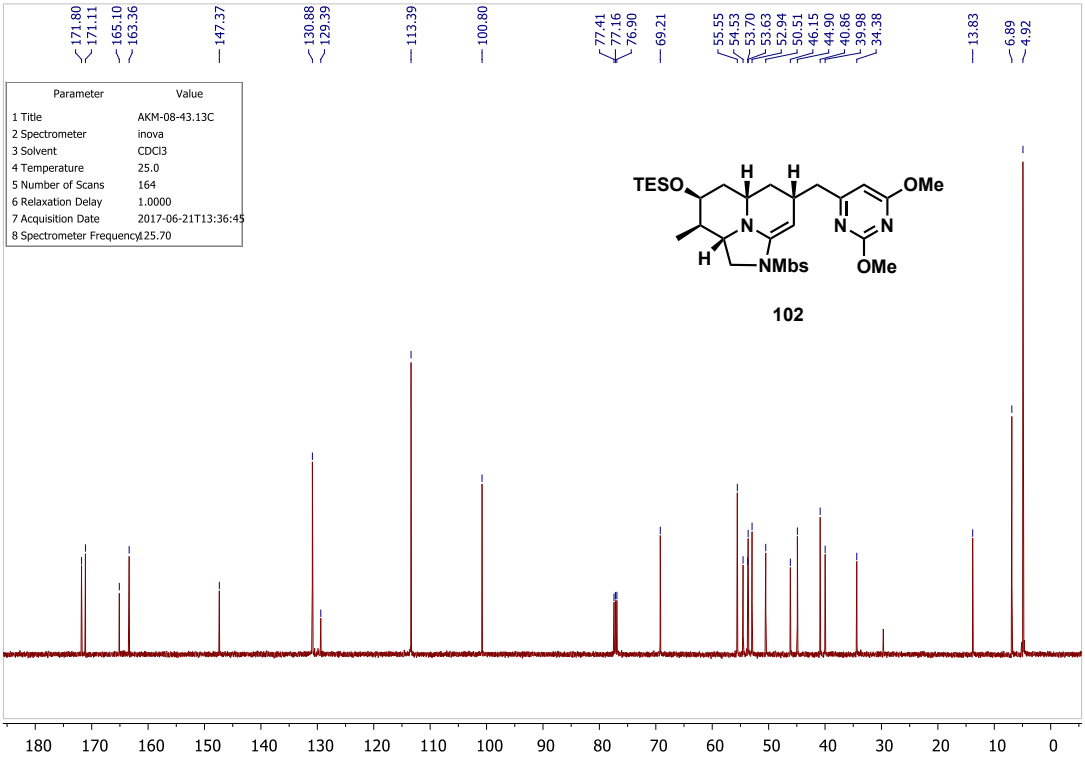

Parameter Value
1 Title AKM-08-52a.15N
2 Spectrometer vnmrs
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 84
6 Relaxation Delay 8.0000
7 Acquisition Date 2017-06-27T12:58:58
8 Spectrometer Frequency 40.51
-148.16
-148.19
-167.10
-167.13
-206.09
-206.21
-253.99
-254.09
-254.11
-254.20
-288.44
-288.46
-288.52
-288.55
TESO
H
H
OMe
15N
15N
15N
15N
H
15NMbs
OMe
102
-80
-100
-120
-140
-160
-180
-200
-220
-240
-260
-280
-300
-320
-340
-360

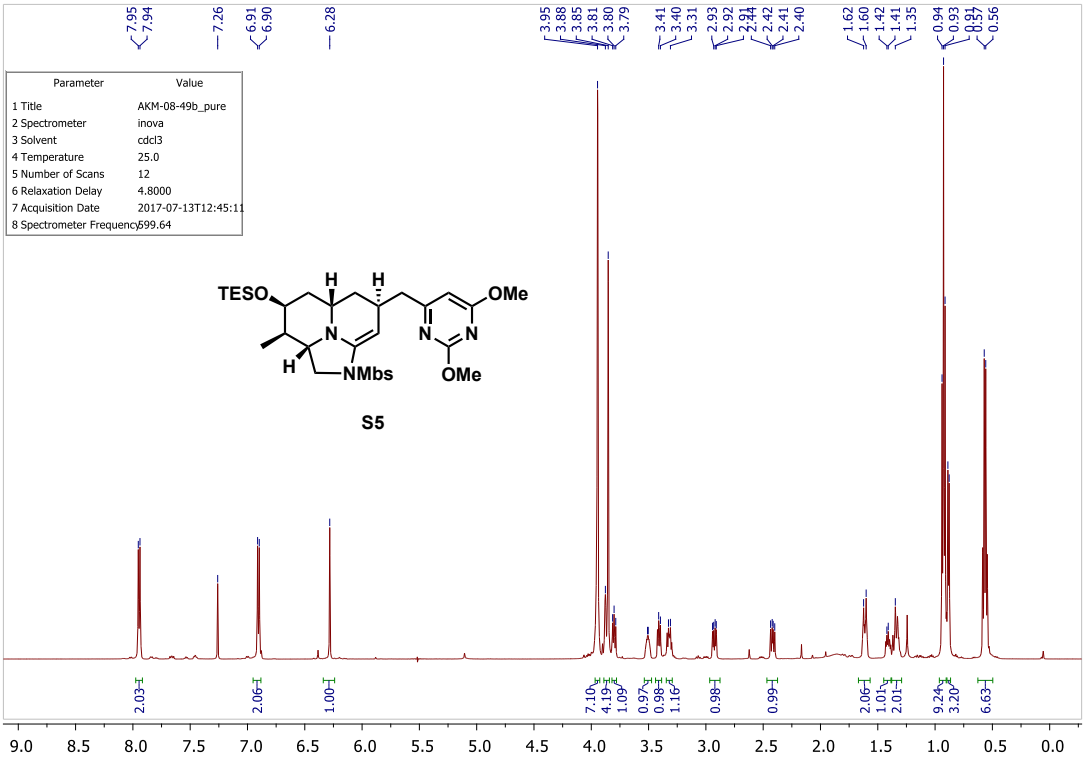

7.92
7.90
6.88
6.86
6.25
3.91
3.91
3.85
3.82
3.77
3.37
3.35
3.31
3.29
2.88
2.88
2.39
2.38
1.57
1.57
1.39
1.38
1.31
0.91
0.89
0.54
0.52
0.51
Parameter Value
1 Title AKM-08-52b_repurified
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 22.0
5 Number of Scans 16
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-07-12T00:41:28
8 Spectrometer Frequency 499.86
TESO
H
H
OMe
15N
15N
15N
15N
H
15NMbs
OMe
S5
2.07
2.11
1.00
7.05
1.09
2.95
1.20
0.97
0.96
1.08
0.98
1.00
1.94
2.95
9.06
3.38
6.19
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

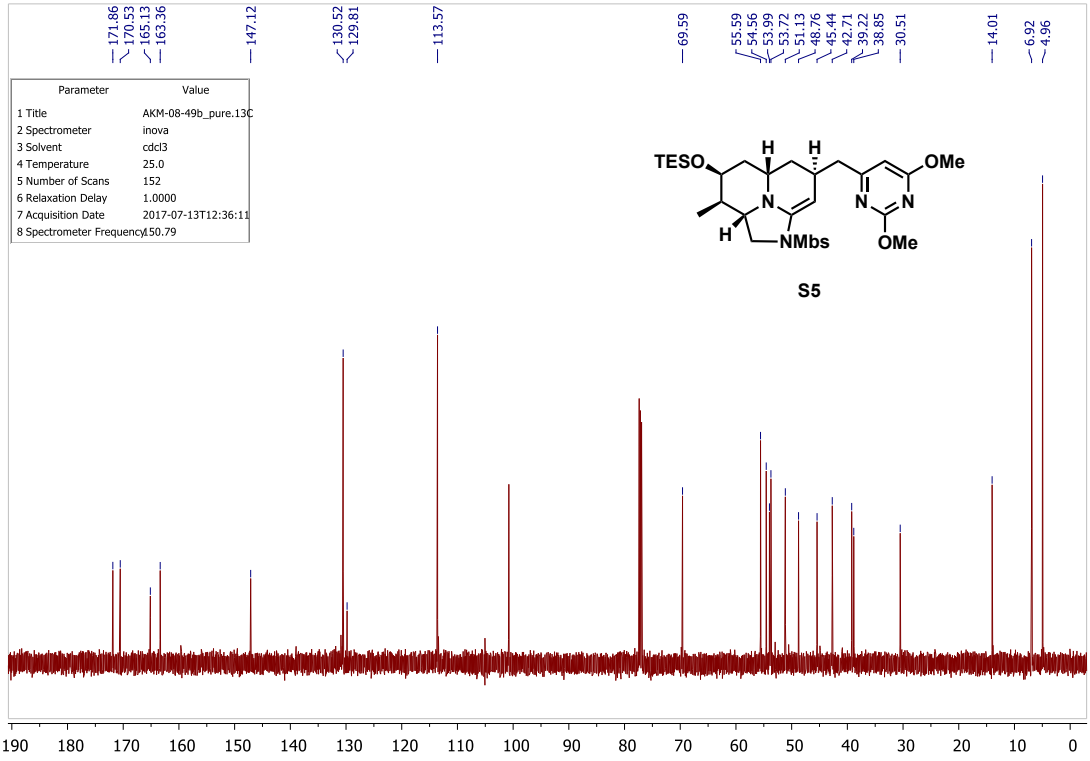

171.88
171.81
170.53
165.16
165.11
165.04
163.35
147.22
147.12
147.04
146.90
130.51
129.84
113.56
100.76
77.41
77.16
76.91
69.59
55.58
54.56
53.99
53.94
53.73
53.70
51.12
48.76
42.72
42.66
39.21
38.84
30.51
29.28
14.01
14.00
6.92
4.95
Parameter Value
1 Title AKM-08-52b_repurified.13C
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 22.0
5 Number of Scans 180
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-07-12T00:45:12
8 Spectrometer Frequency 125.70
TESO
H
H
OMe
15N
15N
15N
15N
H
15NMbs
OMe
S5
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

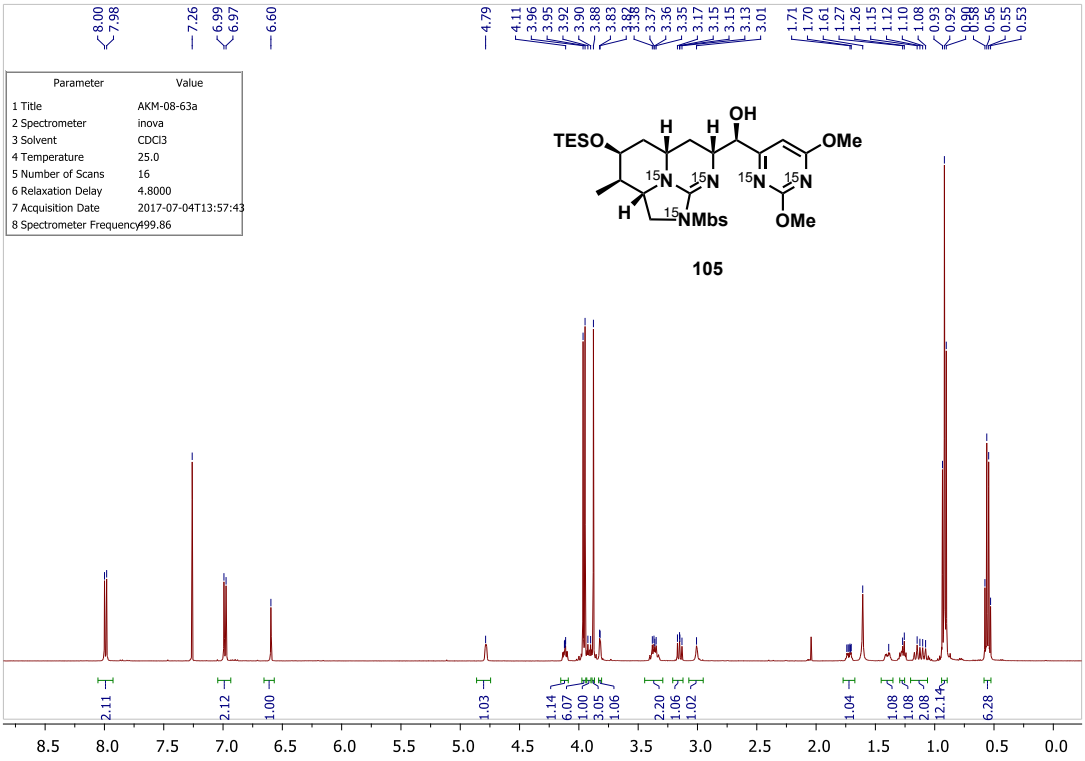

-148.61
-148.64
-166.82
-166.85
-203.61
-203.74
-254.48
-254.57
-254.60
-254.69
-289.33
-289.41
Parameter Value
1 Title AKM-08-52b_repurified.15N
2 Spectrometer vnmrs
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 56
6 Relaxation Delay 8.0000
7 Acquisition Date 2017-07-12T13:02:14
8 Spectrometer Frequency 40.51
TESO
H
H
OMe
15N
15N
15N
15N
H
15NMbs
OMe
S5
-110 -130 -150 -170 -190 -210 -230 -250 -270 -290 -310

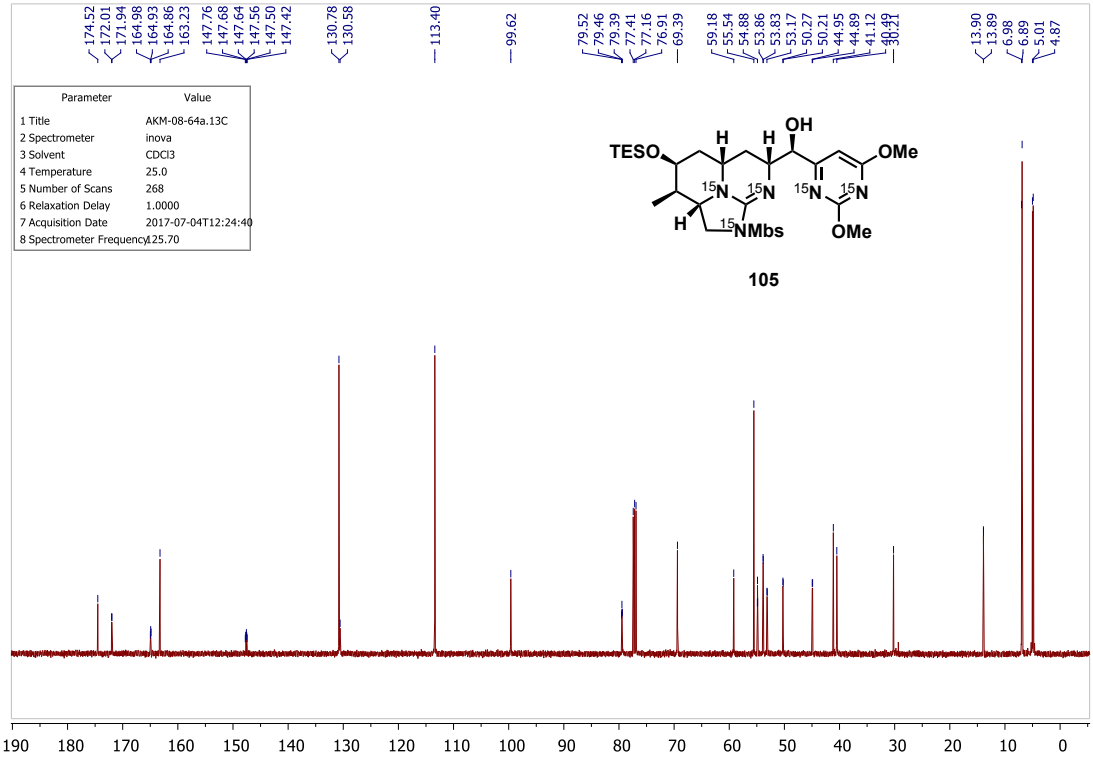

Parameter Value
1 Title AKM-08-43
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 25.0
5 Number of Scans 16
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-06-21T14:35:50
8 Spectrometer Frequency 499.86
TESO
H
H
OMe
N
N
N
H
NMbs
OMe
102

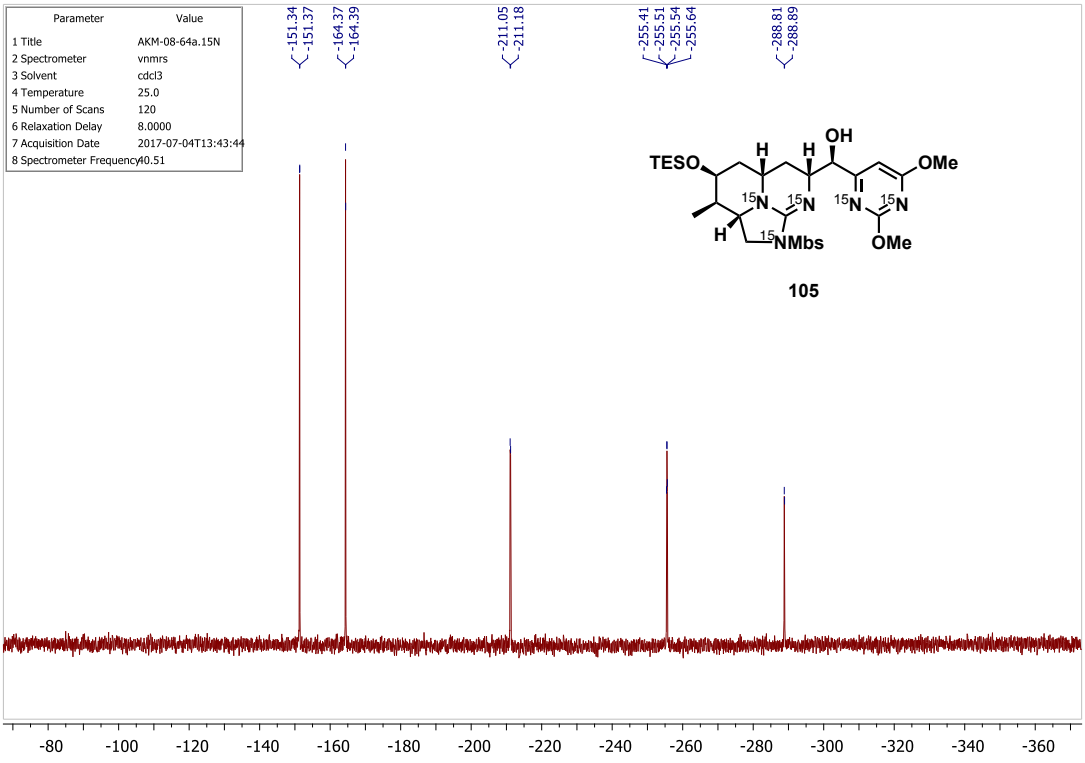

Parameter Value
1 Title AKM-08-43.13C
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 25.0
5 Number of Scans 164
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-06-21T13:36:45
8 Spectrometer Frequency 125.70
TESO
H
H
OMe
N
N
N
H
NMbs
OMe
102

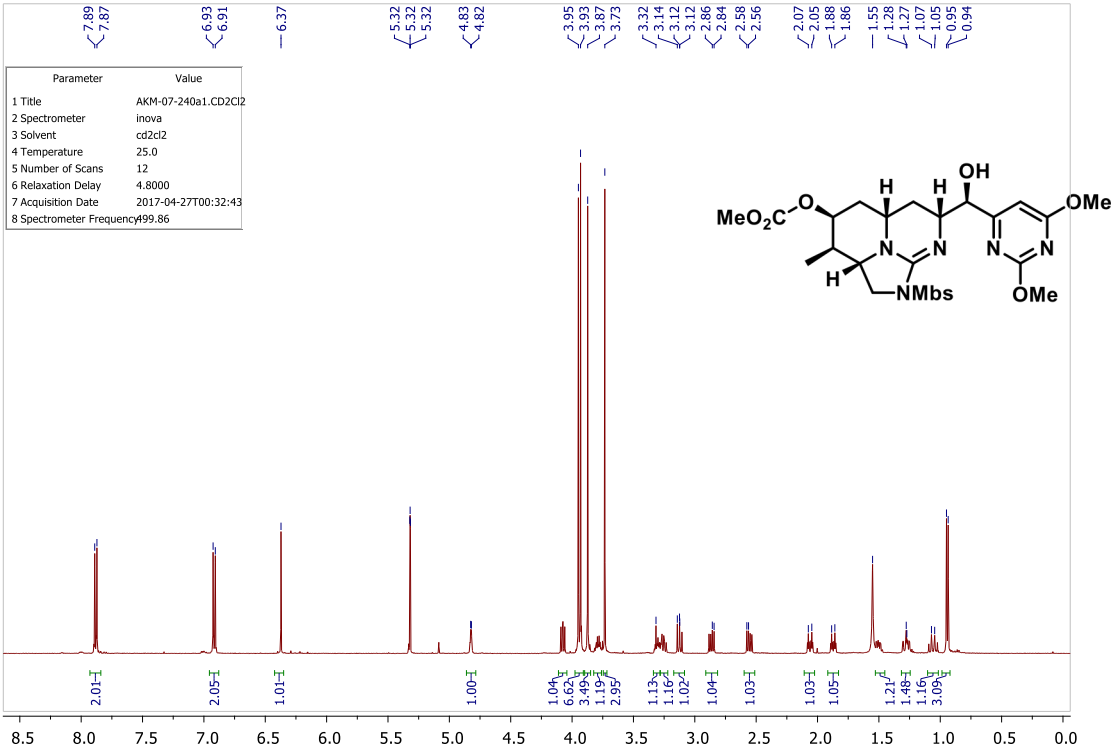

Parameter Value
1 Title AKM-08-49b_pure
2 Spectrometer inova
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 12
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-07-13T12:45:11
8 Spectrometer Frequency 599.64
TESO
OMe
NMbs
OMe
S5

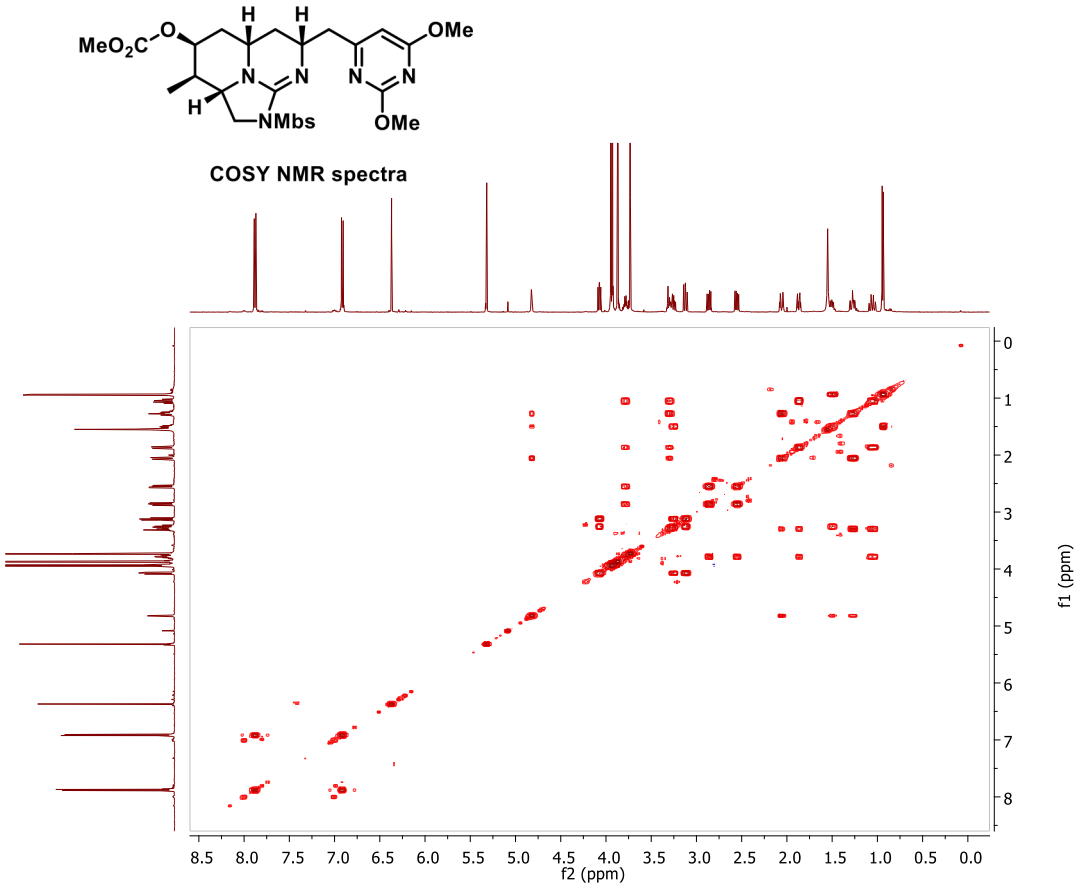

Parameter Value
1 Title AKM-08-49b_pure.13C
2 Spectrometer inova
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 152
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-07-13T12:36:11
8 Spectrometer Frequency 150.79
TESO
OMe
NMbs
OMe
S5

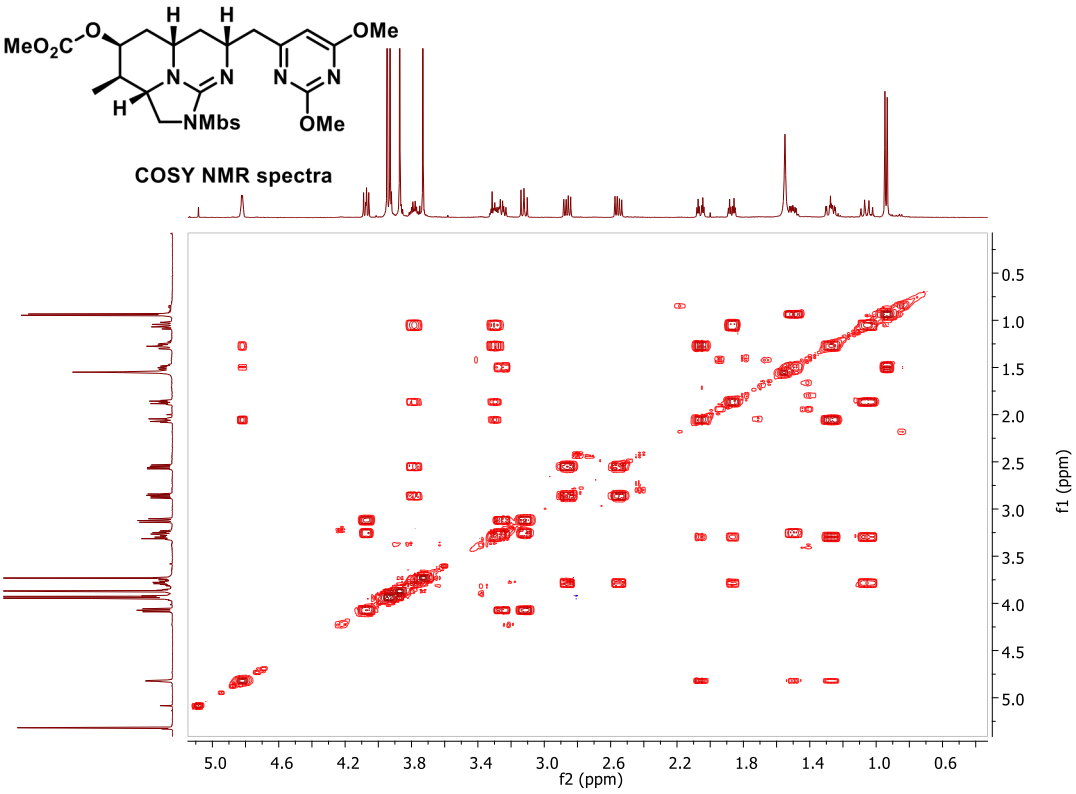

Parameter Value
1 Title AKM-08-63a
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 25.0
5 Number of Scans 16
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-07-04T13:57:43
8 Spectrometer Frequency 499.86
TESO
OH
OMe
OMe
15NMbs
105

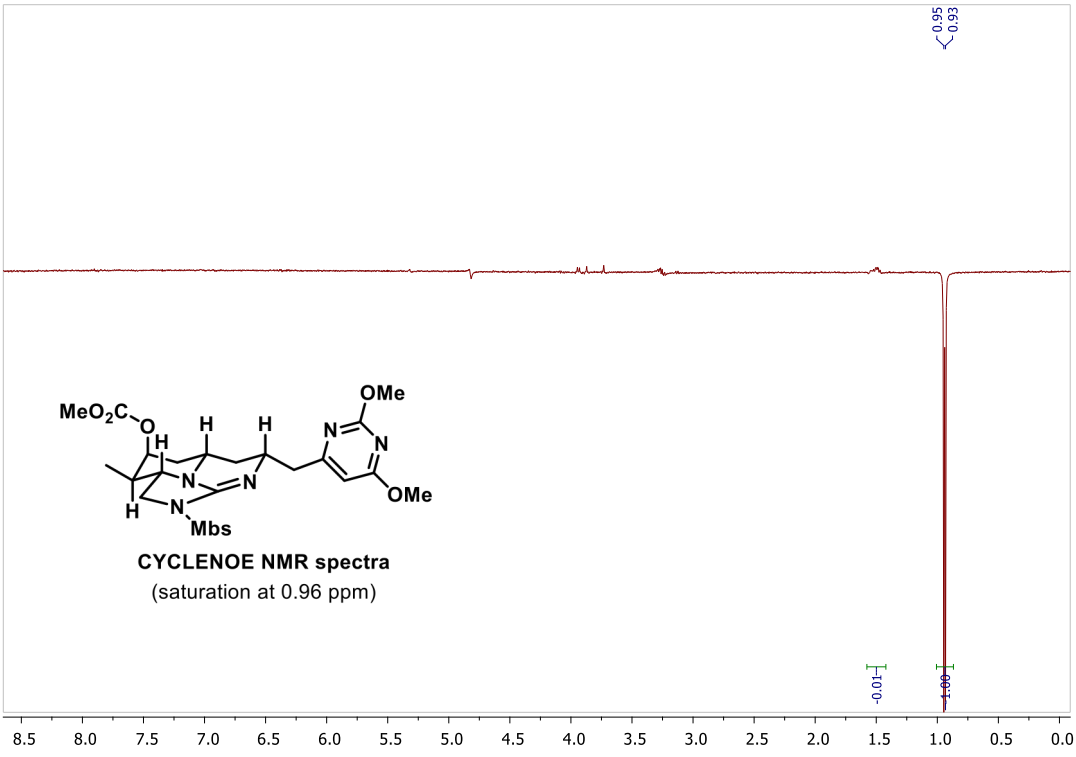

Parameter Value
1 Title AKM-08-64a.13C
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 25.0
5 Number of Scans 268
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-07-04T12:24:40
8 Spectrometer Frequency 125.70
TESO
OH
OMe
OMe
15NMbs
105

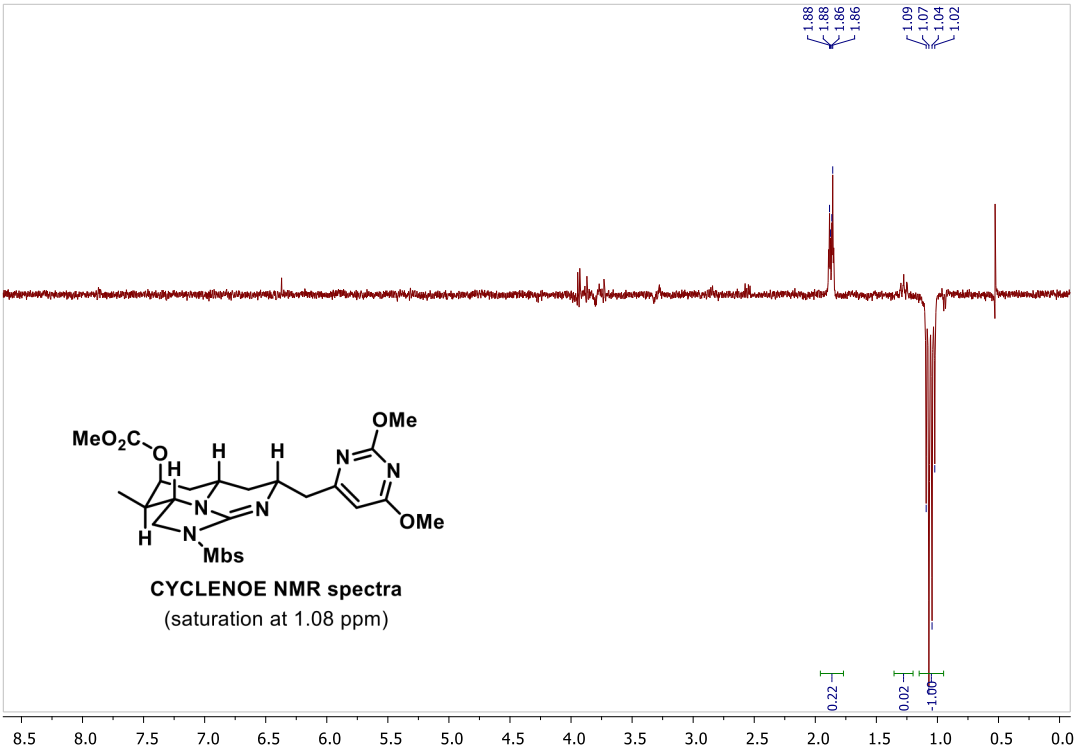

Parameter Value
1 Title AKM-08-64a.15N
2 Spectrometer vnmrs
3 Solvent cdcl3
4 Temperature 25.0
5 Number of Scans 120
6 Relaxation Delay 8.0000
7 Acquisition Date 2017-07-04T13:43:44
8 Spectrometer Frequency 40.51
-151.34
-151.37
-164.37
-164.39
-211.05
-211.18
-255.41
-255.51
-255.54
-255.64
-288.81
-288.89
TESO
OH
H
H
OMe
15N
15N
15N
15N
H
15NMbs
OMe
105
-80
-100
-120
-140
-160
-180
-200
-220
-240
-260
-280
-300
-320
-340
-360


7.89
7.87
6.93
6.91
6.37
5.32
5.32
5.32
4.83
4.82
3.95
3.93
3.87
3.73
3.32
3.14
3.12
3.12
2.86
2.84
2.58
2.56
2.07
2.05
1.88
1.86
1.55
1.28
1.27
1.07
1.05
0.95
0.94
Parameter Value
1 Title AKM-07-240a1.CD2Cl2
2 Spectrometer inova
3 Solvent cd2cl2
4 Temperature 25.0
5 Number of Scans 12
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-04-27T00:32:43
8 Spectrometer Frequency 499.86
MeO2C
O
H
H
OH
OMe
N
N
N
N
H
NMbs
OMe
2.01
2.05
1.01
1.00
1.04
6.62
3.49
1.19
2.95
1.13
1.16
1.02
1.04
1.03
1.03
1.05
1.21
1.48
1.16
3.09
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

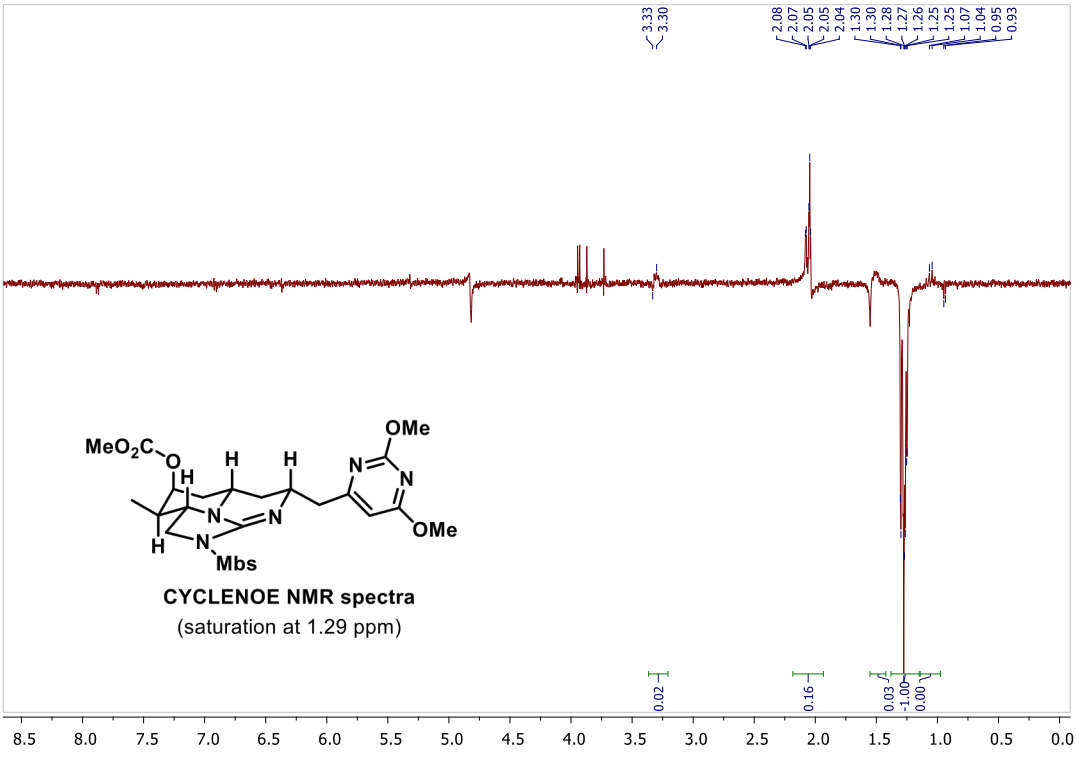

MeO2C
O
H
H
OMe
N
N
N
N
H
NMbs
OMe
COSY NMR spectra
f2 (ppm)
f1 (ppm)


MeO2C
O
H
H
OMe
N
N
N
N
H
NMbs
OMe
COSY NMR spectra
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
f1 (ppm)
5.0
4.6
4.2
3.8
3.4
3.0
2.6
2.2
1.8
1.4
1.0
0.6
f2 (ppm)

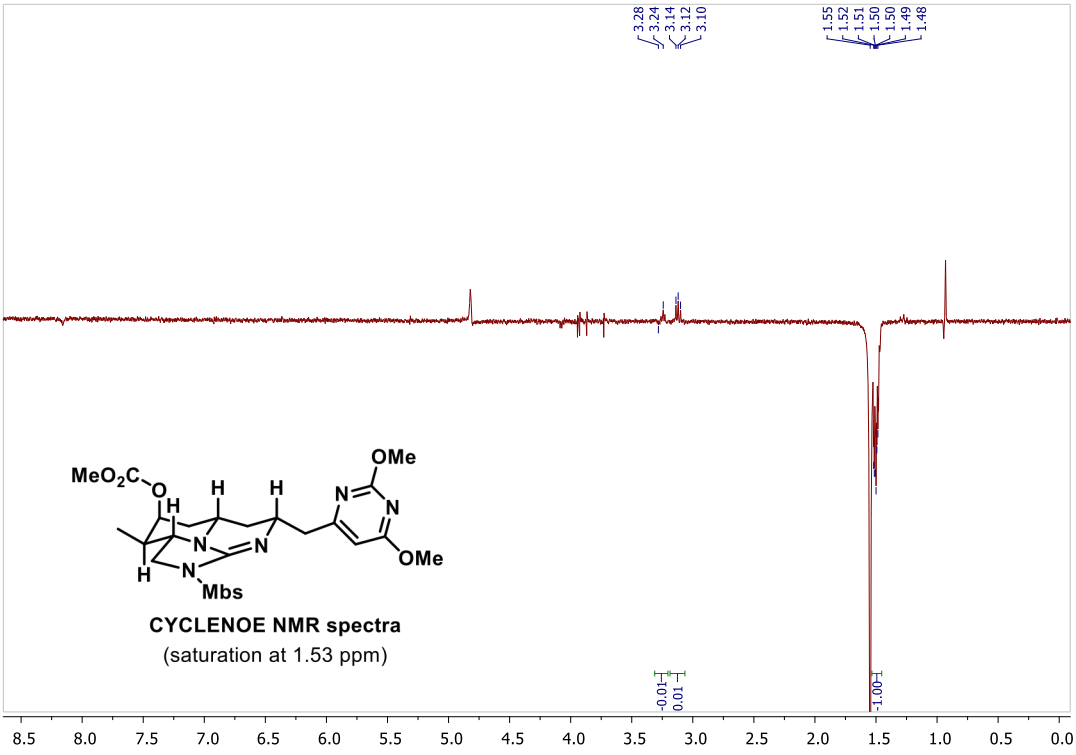

0.95
0.93
MeO2C
O
H
H
H
OMe
N
N
N
N
H
N
Mbs
OMe
CYCLENOE NMR spectra
(saturation at 0.96 ppm)
-0.01
1.00
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0




**CYCLENOE NMR spectra**
(saturation at 1.08 ppm)

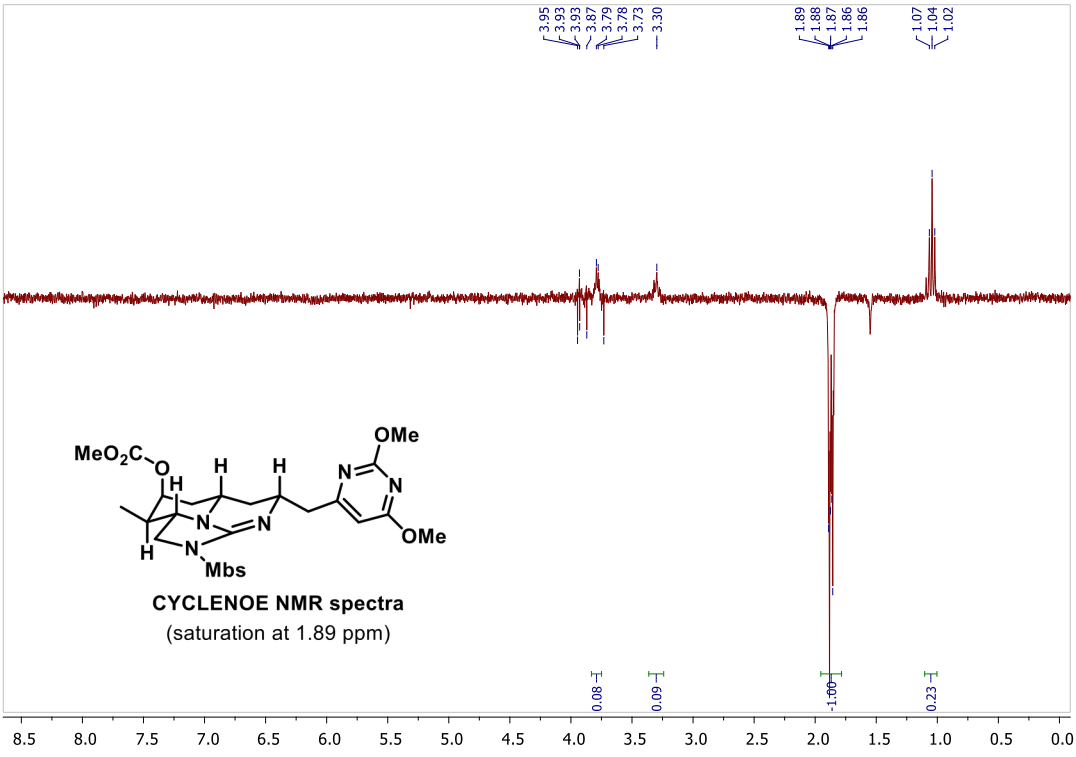



**CYCLENOE NMR spectra**
(saturation at 1.29 ppm)



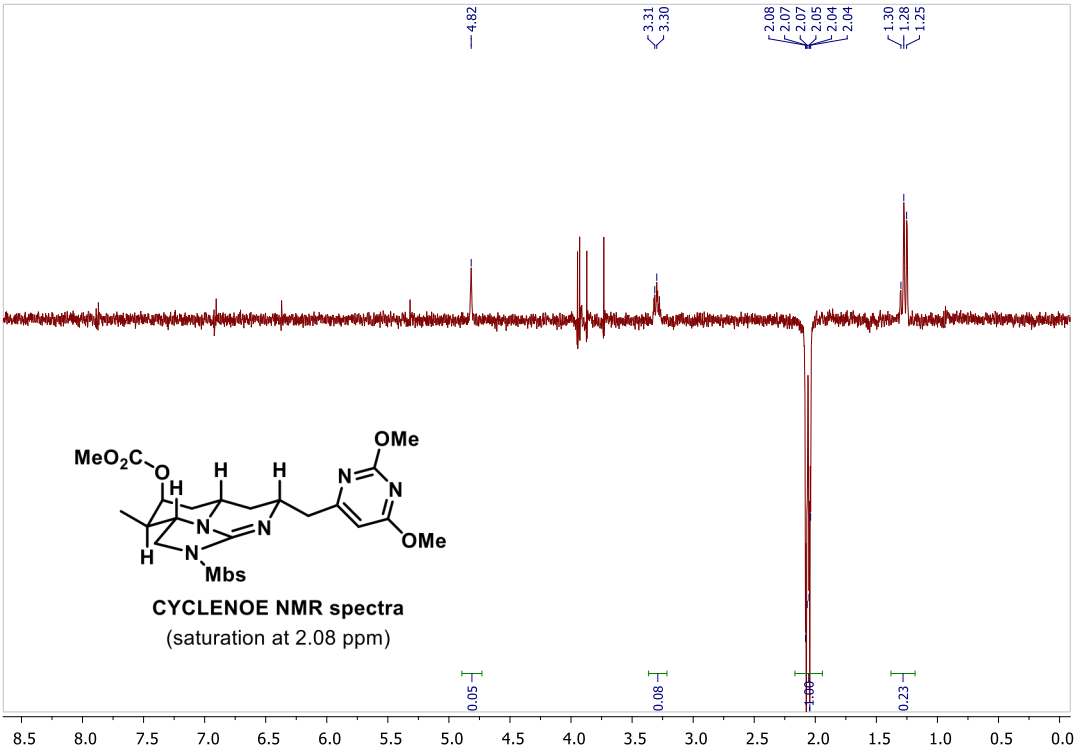

**CYCLENOE NMR spectra**

(saturation at 1.53 ppm)

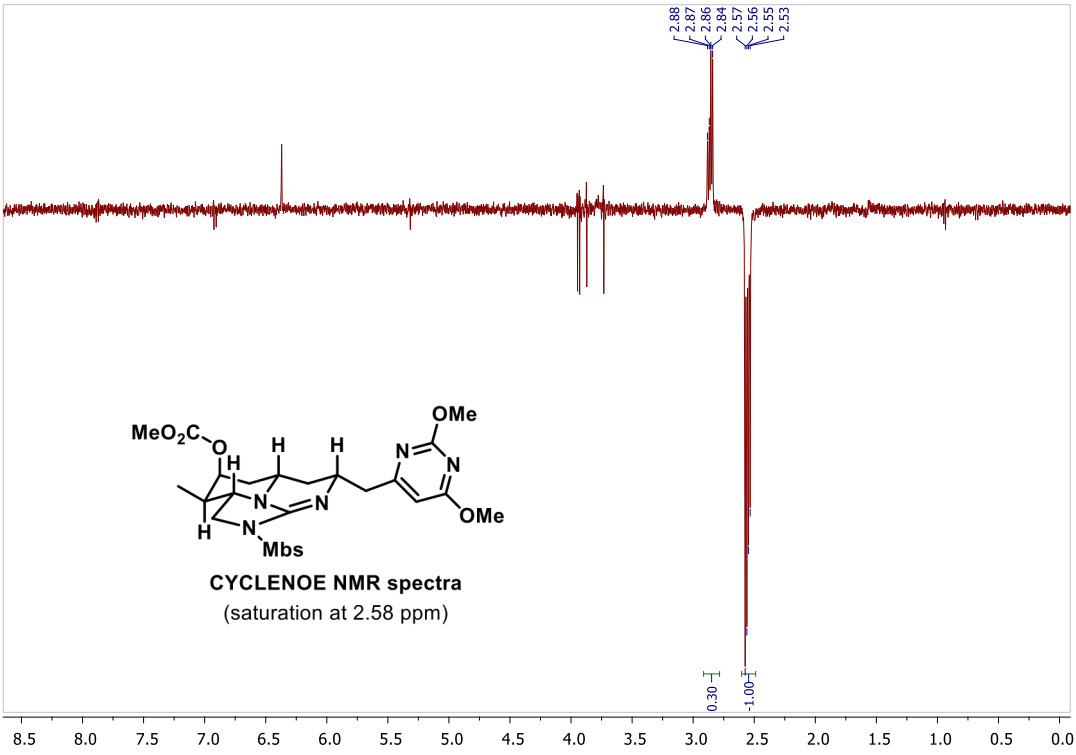



**CYCLENOE NMR spectra**

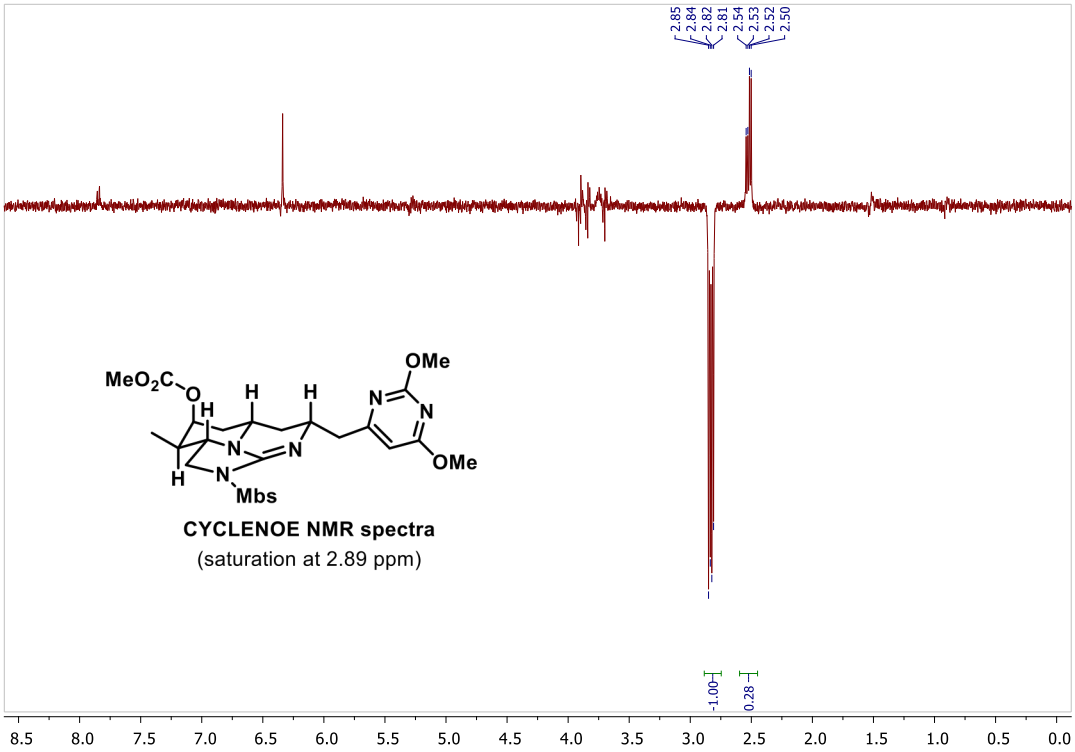
(saturation at 1.89 ppm)



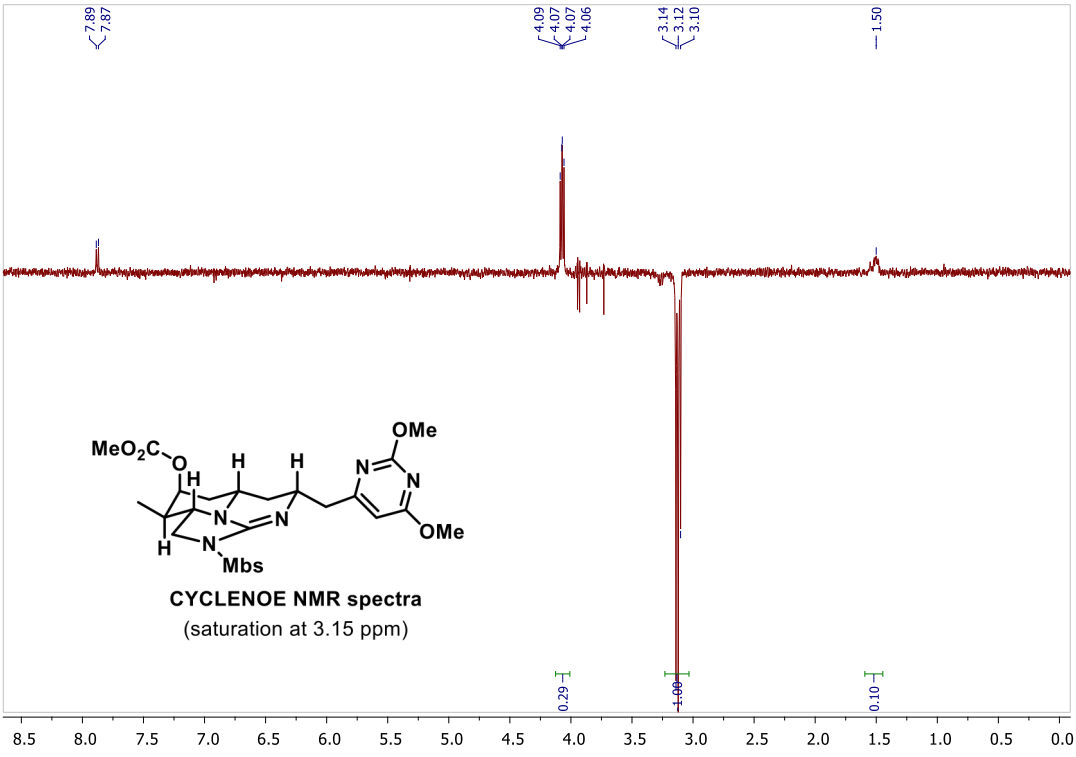

**CYCLENOE NMR spectra**
(saturation at 2.08 ppm)



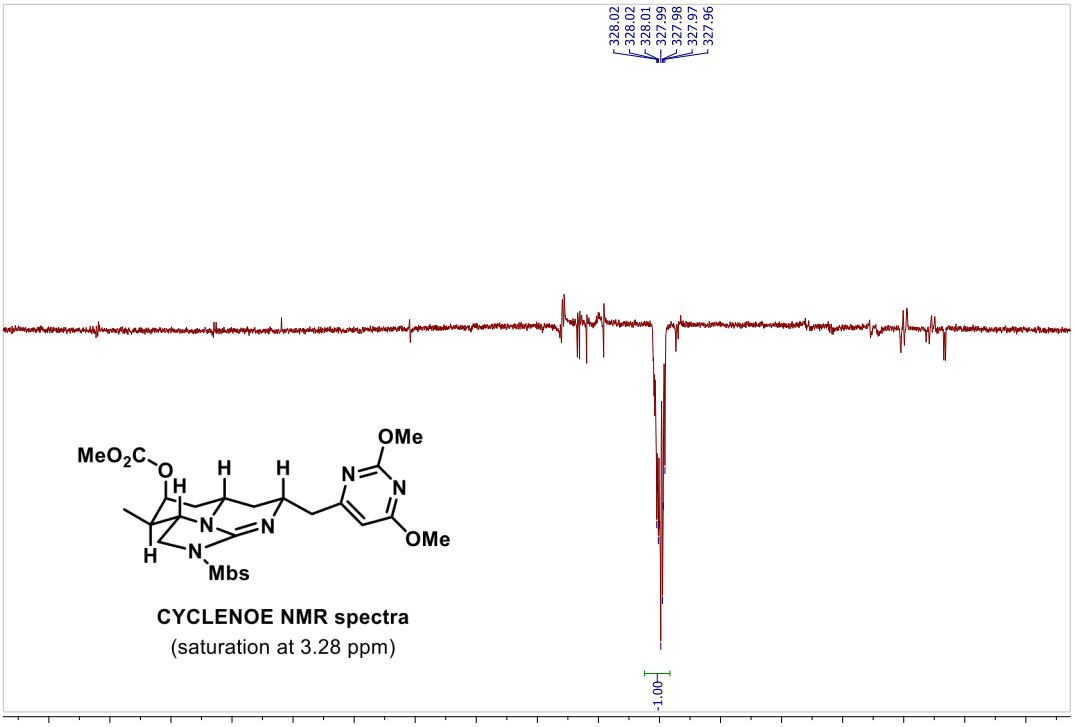

**CYCLENOE NMR spectra**
(saturation at 2.58 ppm)



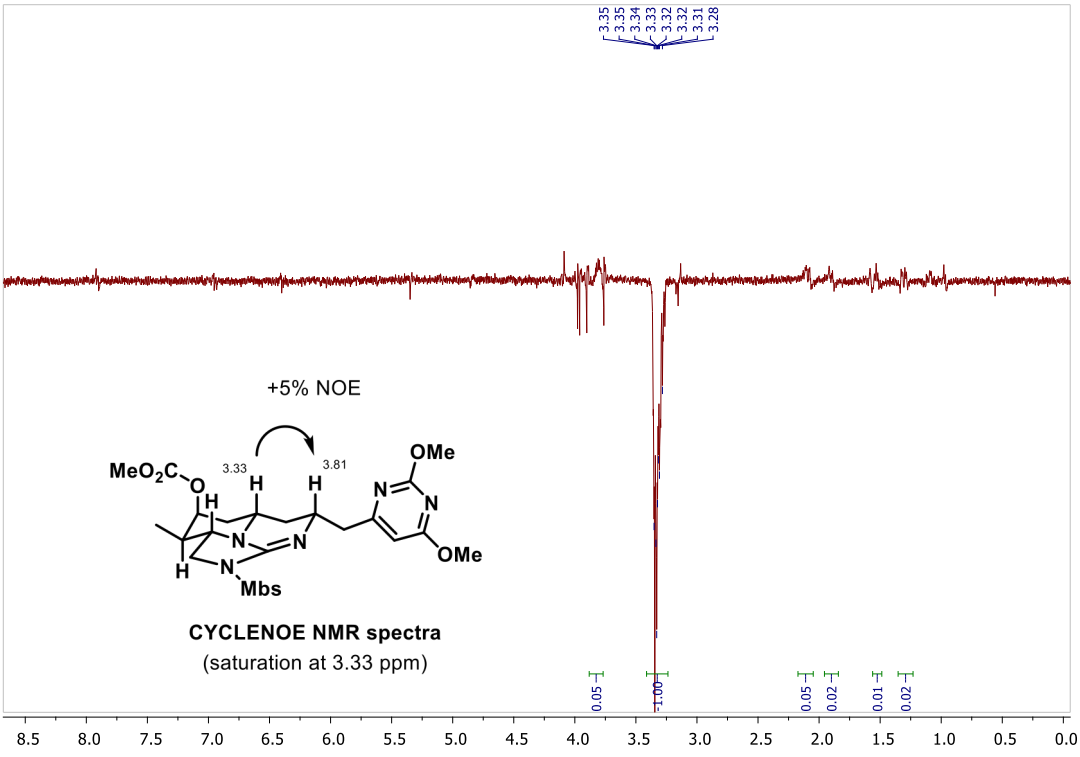

**CYCLENOE NMR spectra**
(saturation at 2.89 ppm)



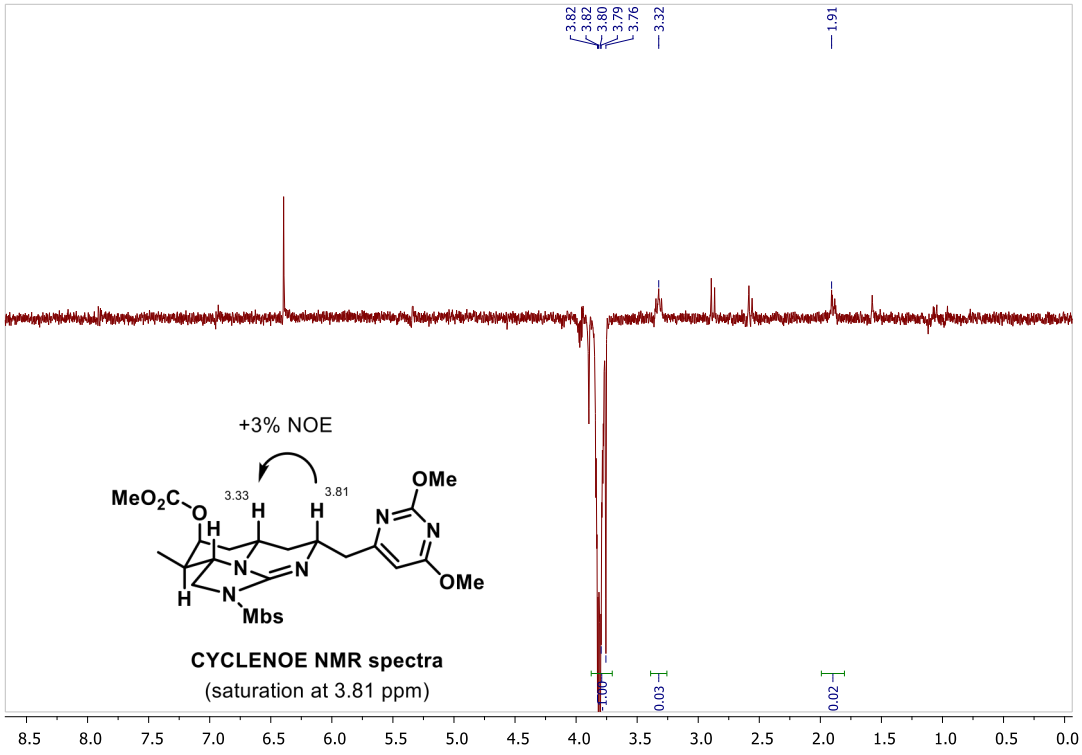

**CYCLENOE NMR spectra**
(saturation at 3.15 ppm)

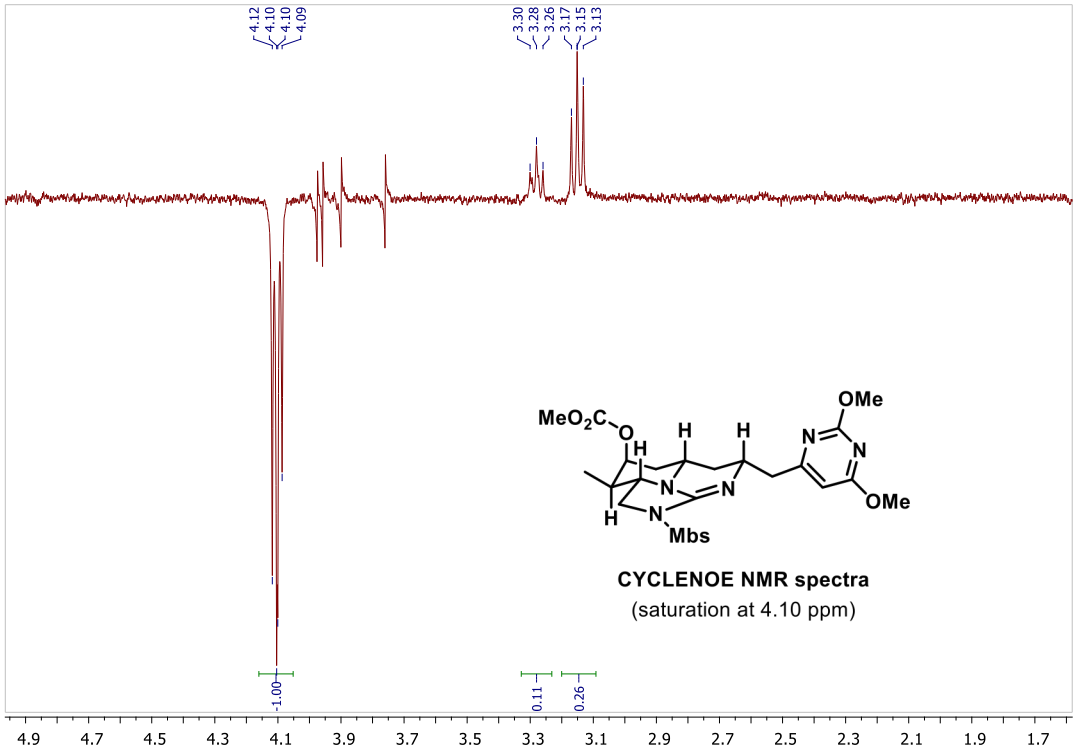


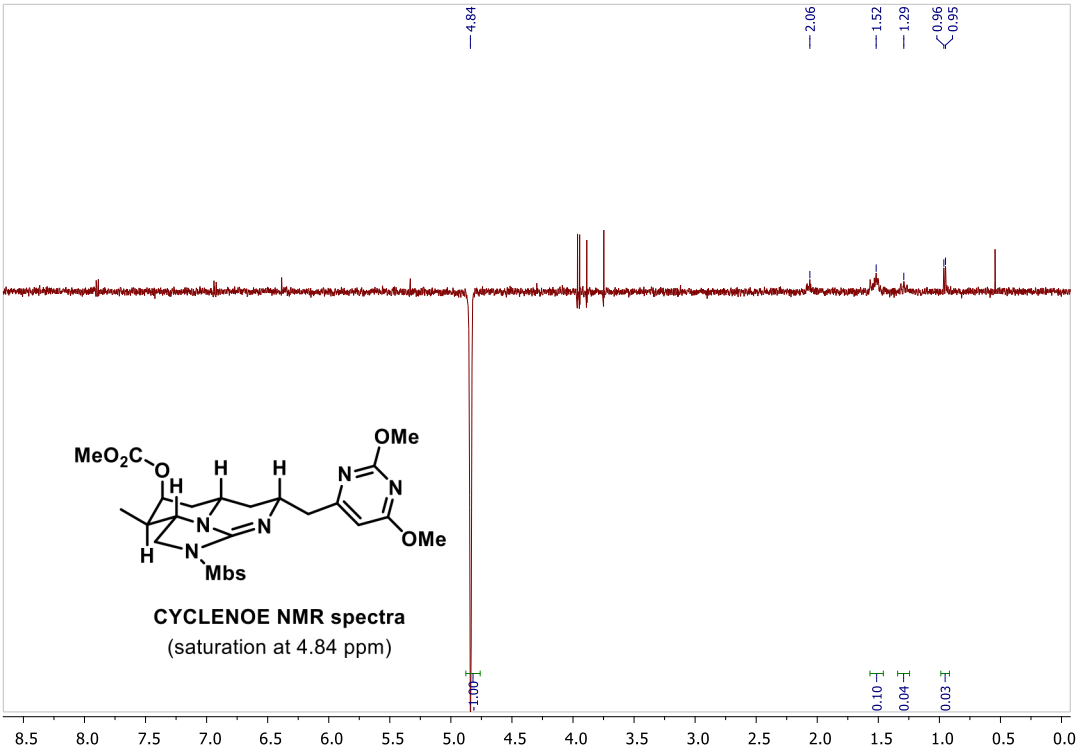

**CYCLENOE NMR spectra**
(saturation at 3.28 ppm)

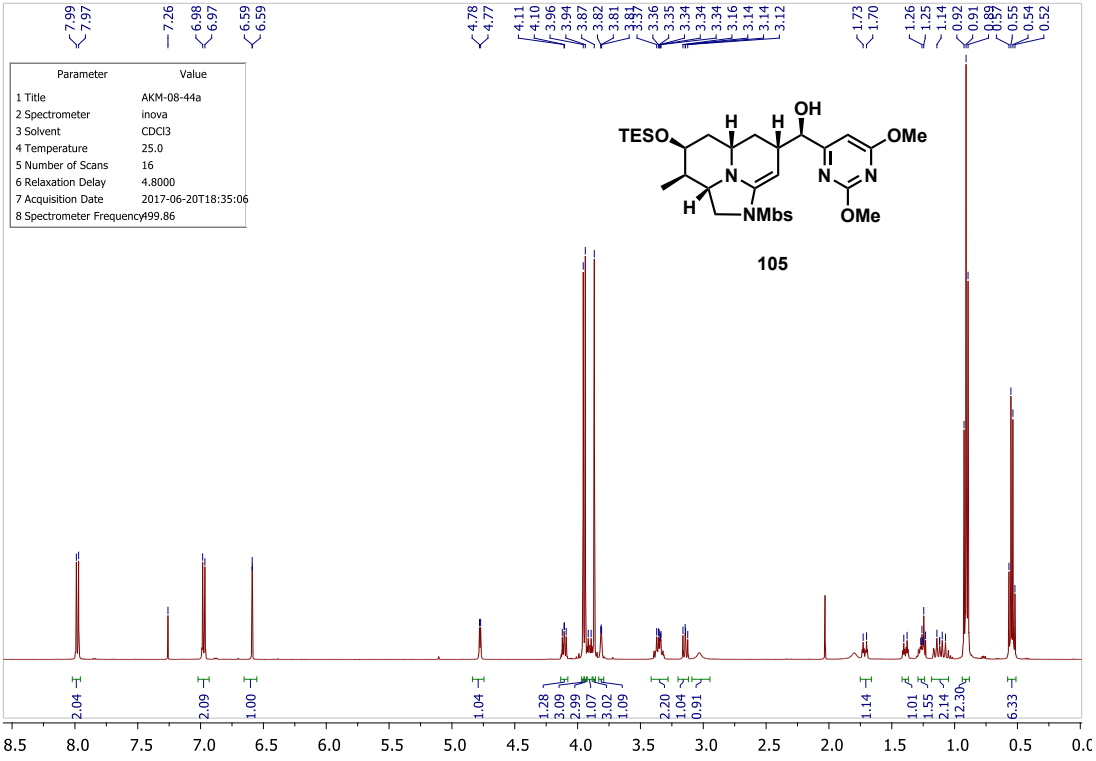


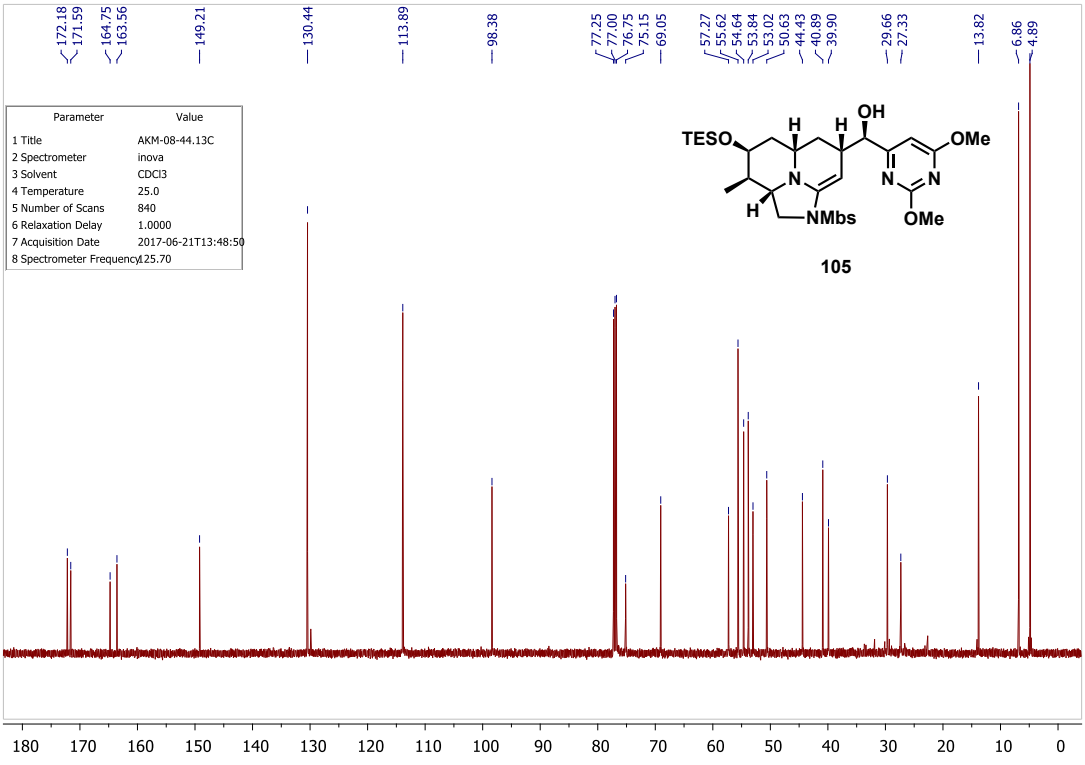

**CYCLENOE NMR spectra**
(saturation at 3.33 ppm)

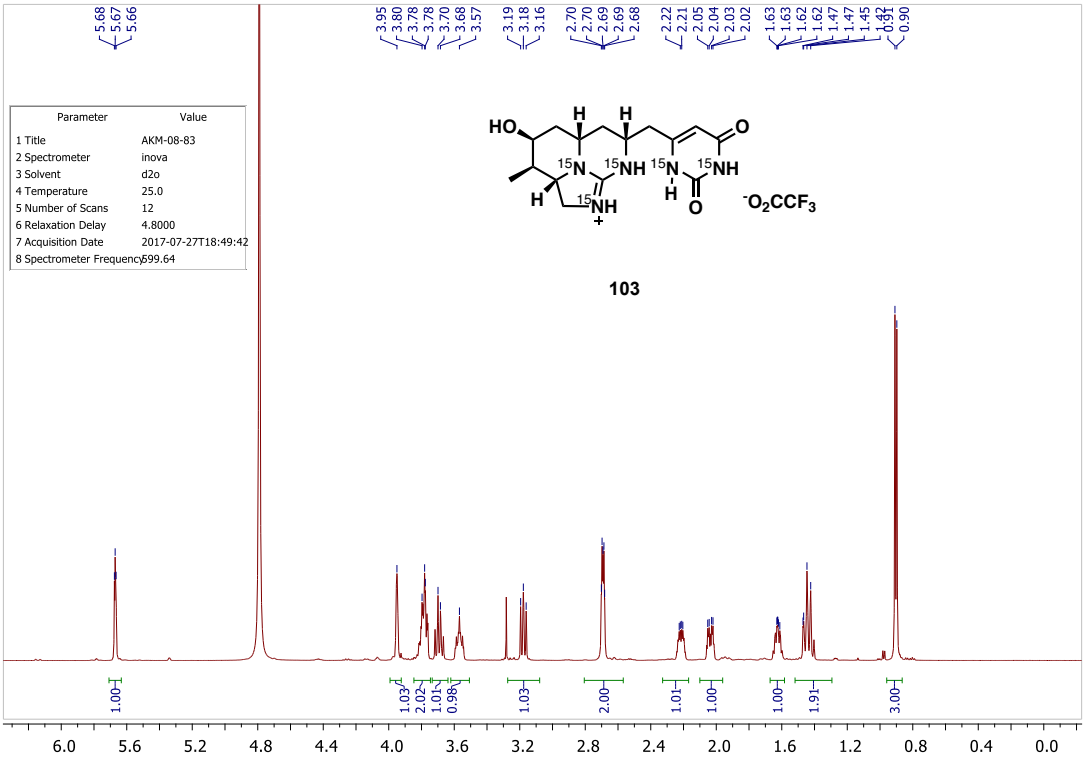

3.82
3.82
3.80
3.79
3.76
3.32
1.91
+3% NOE
3.33
3.81
MeO2C
OMe
OMe
Mbs
CYCLENOE NMR spectra
(saturation at 3.81 ppm)
1.00
0.03
0.02
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

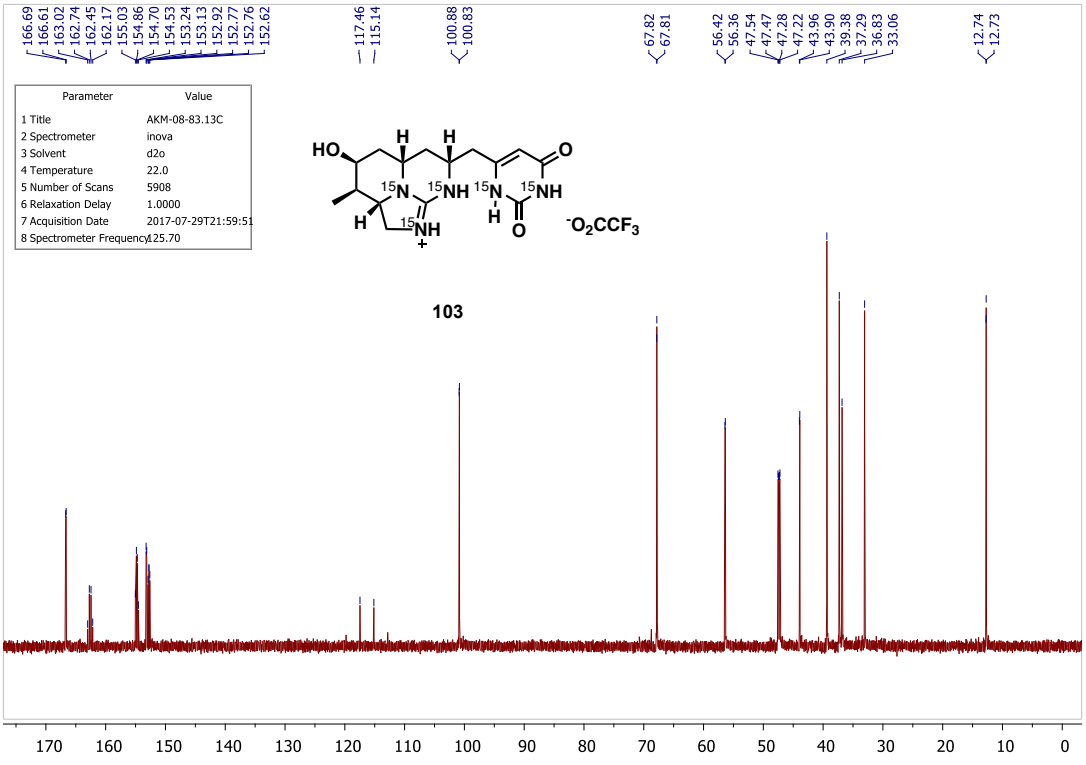

4.12
4.10
4.10
4.09
3.30
3.28
3.26
3.17
3.15
3.13
MeO2C
OMe
OMe
Mbs
CYCLENOE NMR spectra
(saturation at 4.10 ppm)
1.00
0.11
0.26
4.9 4.7 4.5 4.3 4.1 3.9 3.7 3.5 3.3 3.1 2.9 2.7 2.5 2.3 2.1 1.9 1.7

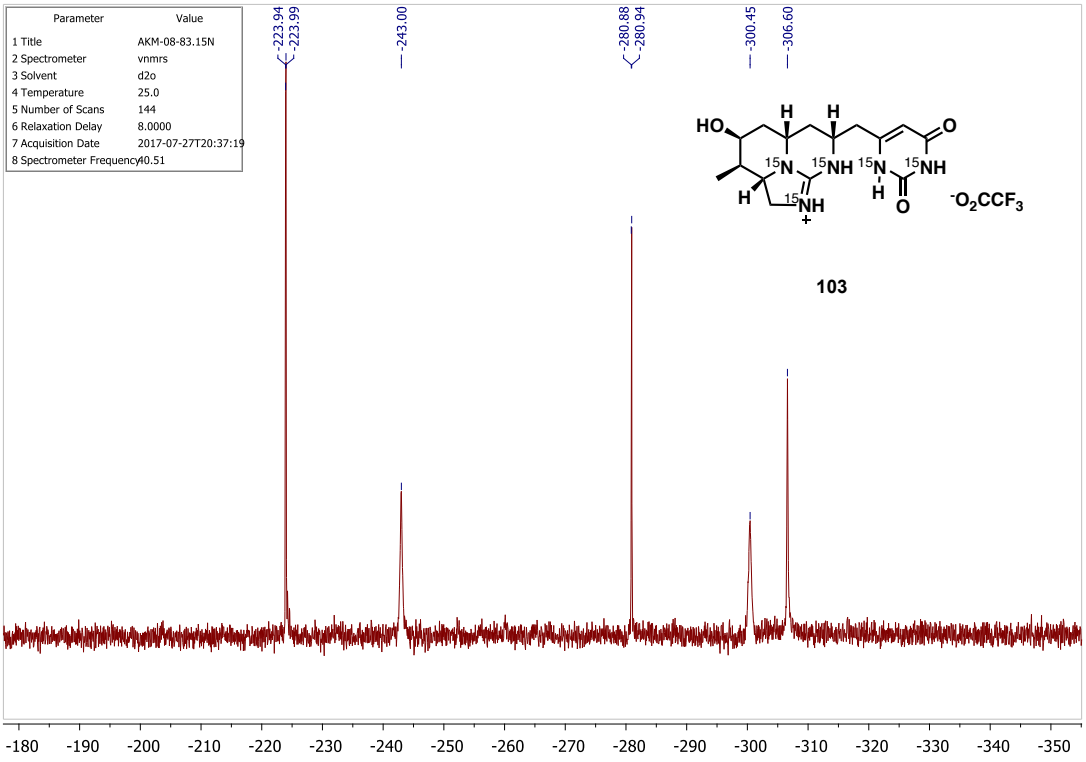

4.84
2.06
1.52
1.29
0.96
0.95
MeO2C
OMe
OMe
Mbs
CYCLENOE NMR spectra
(saturation at 4.84 ppm)
1.00
0.10
0.04
0.03

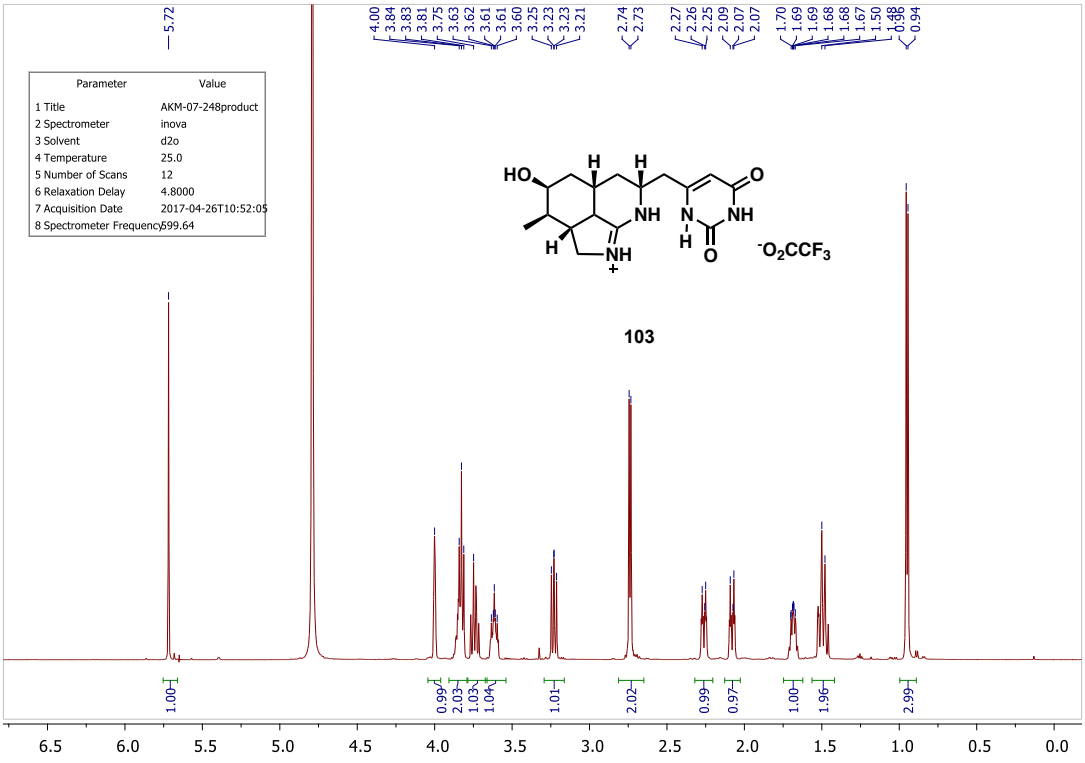

Parameter Value
1 Title AKM-08-44a
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 25.0
5 Number of Scans 16
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-06-20T18:35:06
8 Spectrometer Frequency 499.86
TESO
OH
OMe
OMe
NMbs
105

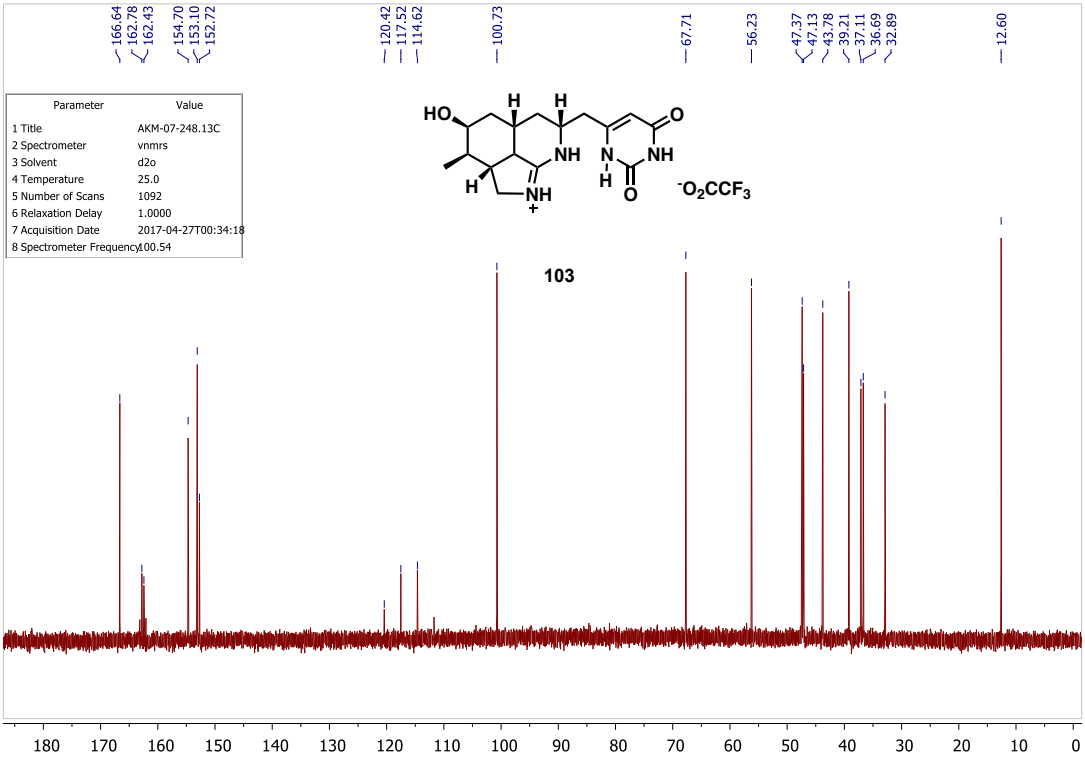

172.18
171.59
164.75
163.56
149.21
130.44
113.89
98.38
77.25
77.00
76.75
75.15
69.05
57.27
55.62
54.64
53.84
53.02
50.63
44.43
40.89
39.90
29.66
27.33
13.82
6.86
4.89
Parameter Value
1 Title AKM-08-44.13C
2 Spectrometer inova
3 Solvent CDCl3
4 Temperature 25.0
5 Number of Scans 840
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-06-21T13:48:50
8 Spectrometer Frequency 125.70
TESO
H
H
OH
OMe
N
N
N
H
NMbs
OMe
105
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

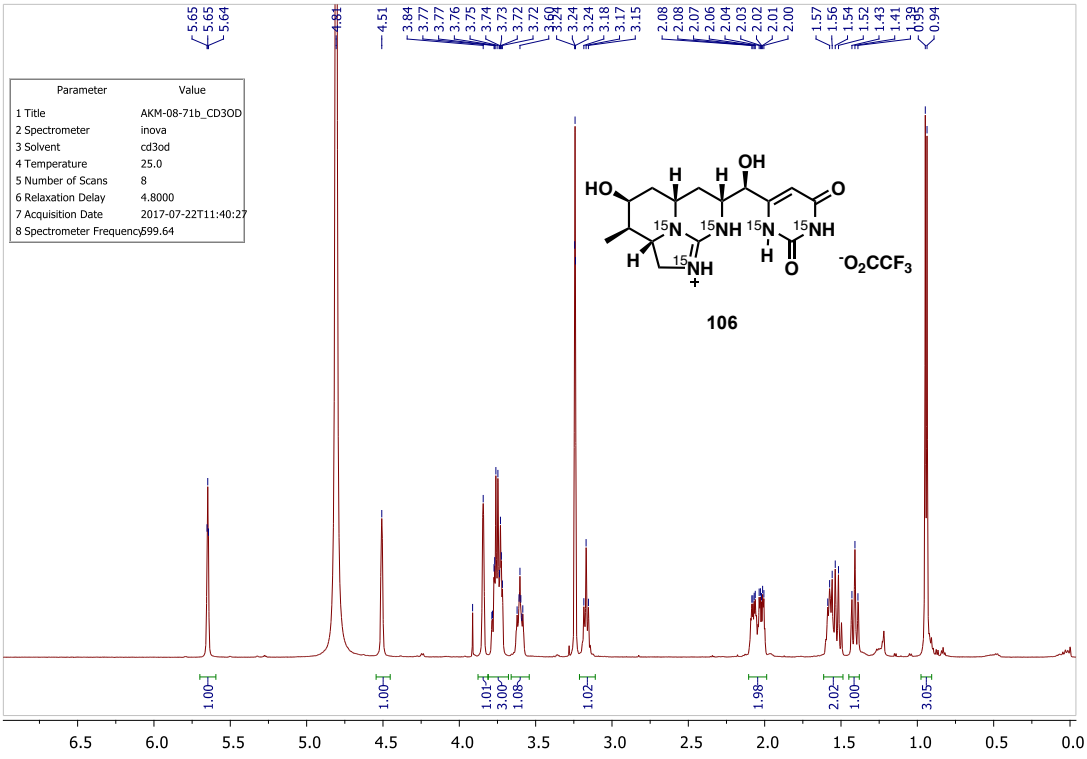

5.68
5.67
5.66
3.95
3.80
3.78
3.78
3.70
3.68
3.57
3.19
3.18
3.16
2.70
2.70
2.69
2.69
2.68
2.22
2.21
2.05
2.04
2.03
2.02
1.63
1.63
1.62
1.62
1.47
1.47
1.45
0.92
0.91
0.90
Parameter Value
1 Title AKM-08-83
2 Spectrometer inova
3 Solvent d2o
4 Temperature 25.0
5 Number of Scans 12
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-07-27T18:49:42
8 Spectrometer Frequency 599.64
HO
H
H
O
15N
15NH
15N
15NH
H
15NH
+
H
N
O
-O2CCF3
103
1.00
1.03
2.02
1.01
0.98
1.03
2.00
1.01
1.00
1.00
1.91
3.00
6.0 5.6 5.2 4.8 4.4 4.0 3.6 3.2 2.8 2.4 2.0 1.6 1.2 0.8 0.4 0.0

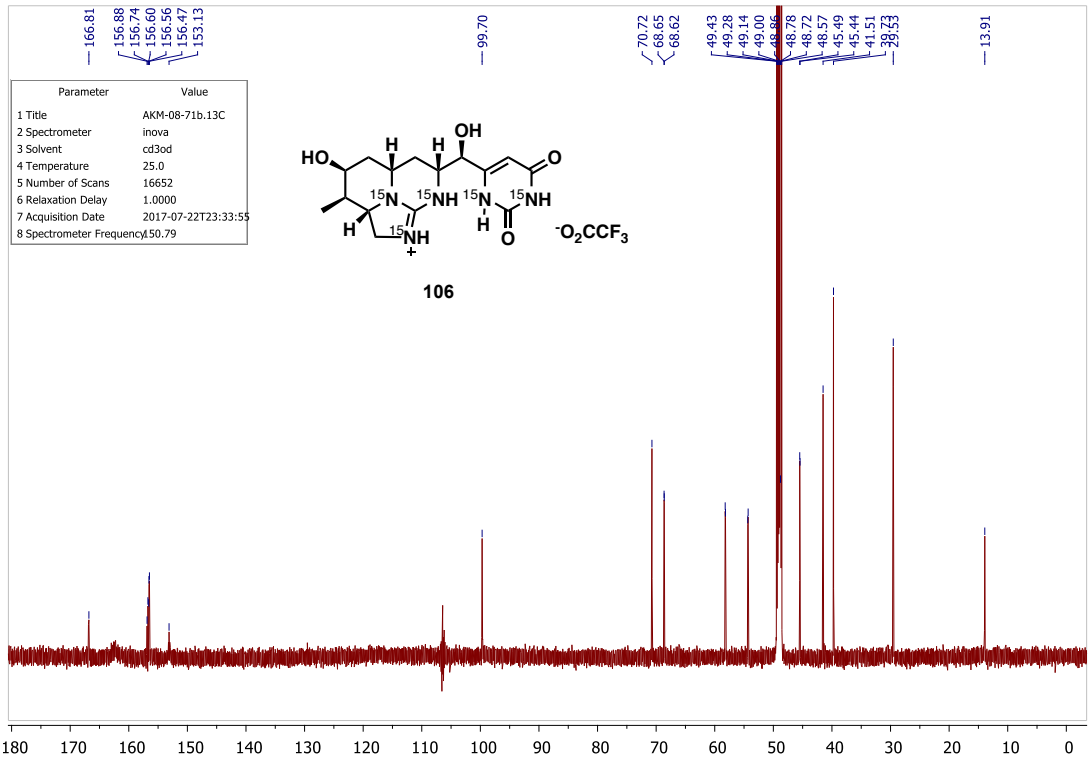

166.69
166.61
163.02
162.74
162.45
162.17
155.03
154.86
154.70
154.53
153.24
153.13
152.92
152.77
152.76
152.62
117.46
115.14
100.88
100.83
67.82
67.81
56.42
56.36
47.54
47.47
47.28
47.22
43.96
43.90
39.38
37.29
36.83
33.06
12.74
12.73
Parameter Value
1 Title AKM-08-83.13C
2 Spectrometer inova
3 Solvent d2o
4 Temperature 22.0
5 Number of Scans 5908
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-07-29T21:59:51
8 Spectrometer Frequency 125.70
HO
H
H
O
15N
15NH
15N
15NH
H
15NH
H
O
-O2CCF3
103
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

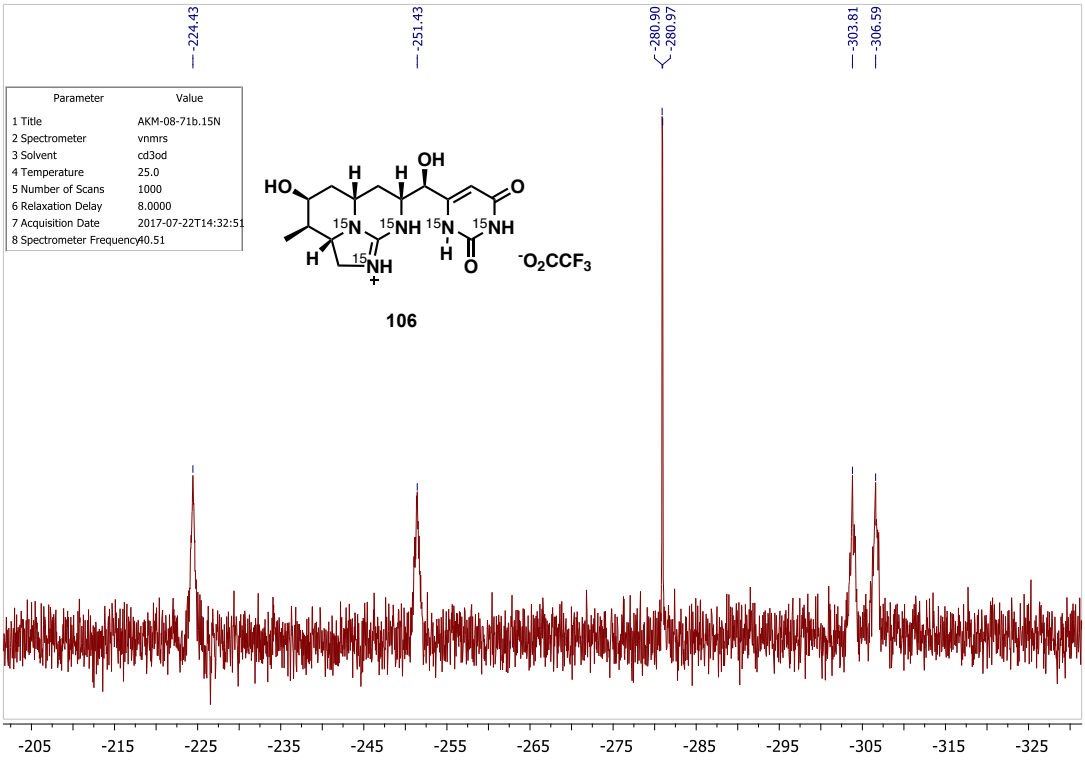

-223.94
-223.99
-243.00
-280.88
-280.94
-300.45
-306.60
Parameter Value
1 Title AKM-08-83.15N
2 Spectrometer vnmrs
3 Solvent d2o
4 Temperature 25.0
5 Number of Scans 144
6 Relaxation Delay 8.0000
7 Acquisition Date 2017-07-27T20:37:19
8 Spectrometer Frequency 40.51
HO
H
H
O
15N
15NH
15N
15NH
H
15NH
H
O
-O2CCF3
103
-180 -190 -200 -210 -220 -230 -240 -250 -260 -270 -280 -290 -300 -310 -320 -330 -340 -350

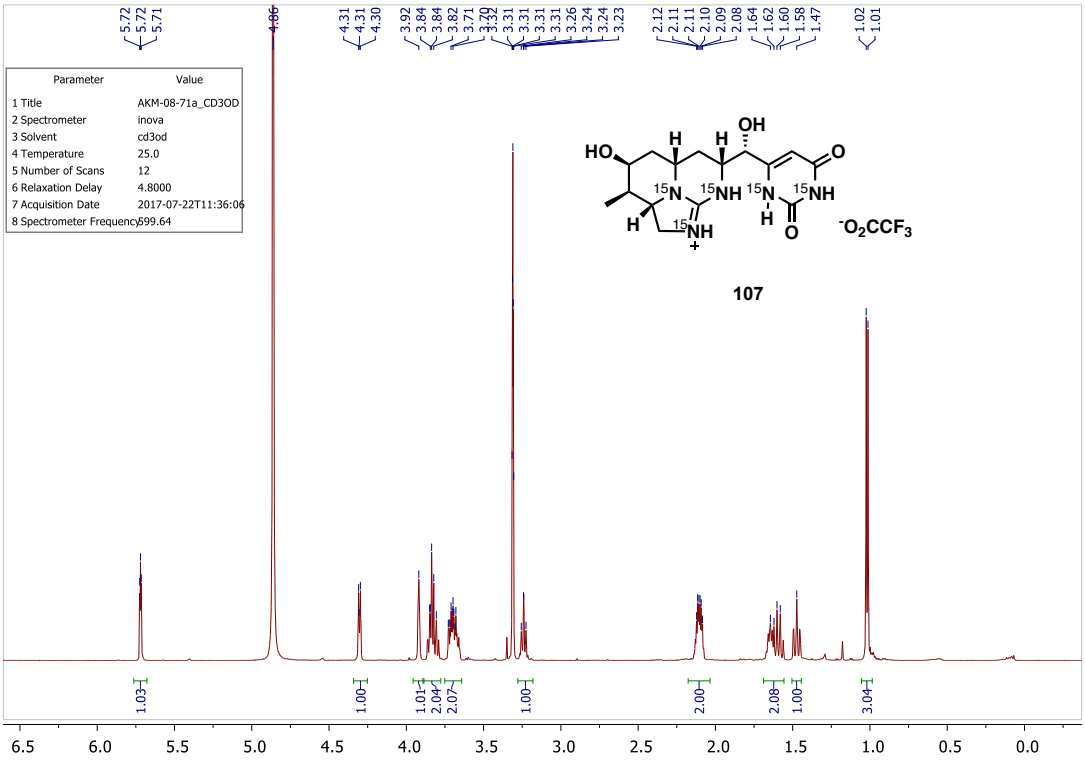

Parameter Value
1 Title AKM-07-248product
2 Spectrometer inova
3 Solvent d2o
4 Temperature 25.0
5 Number of Scans 12
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-04-26T10:52:05
8 Spectrometer Frequency 599.64
5.72
4.00 3.84 3.83 3.81 3.75 3.63 3.62 3.61 3.61 3.60 3.25 3.23 3.23 3.21
2.74 2.73
2.27 2.26 2.25 2.09 2.07 2.07
1.70 1.69 1.69 1.68 1.68 1.67 1.50 0.98 0.94
HO
H
H
NH
N
H
NH
O
O
H
NH
+
$^{-}O_2CCF_3$
103
1.00 0.99 2.03 1.03 1.04 1.01 2.02 0.99 0.97 1.00 1.96 2.99
6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

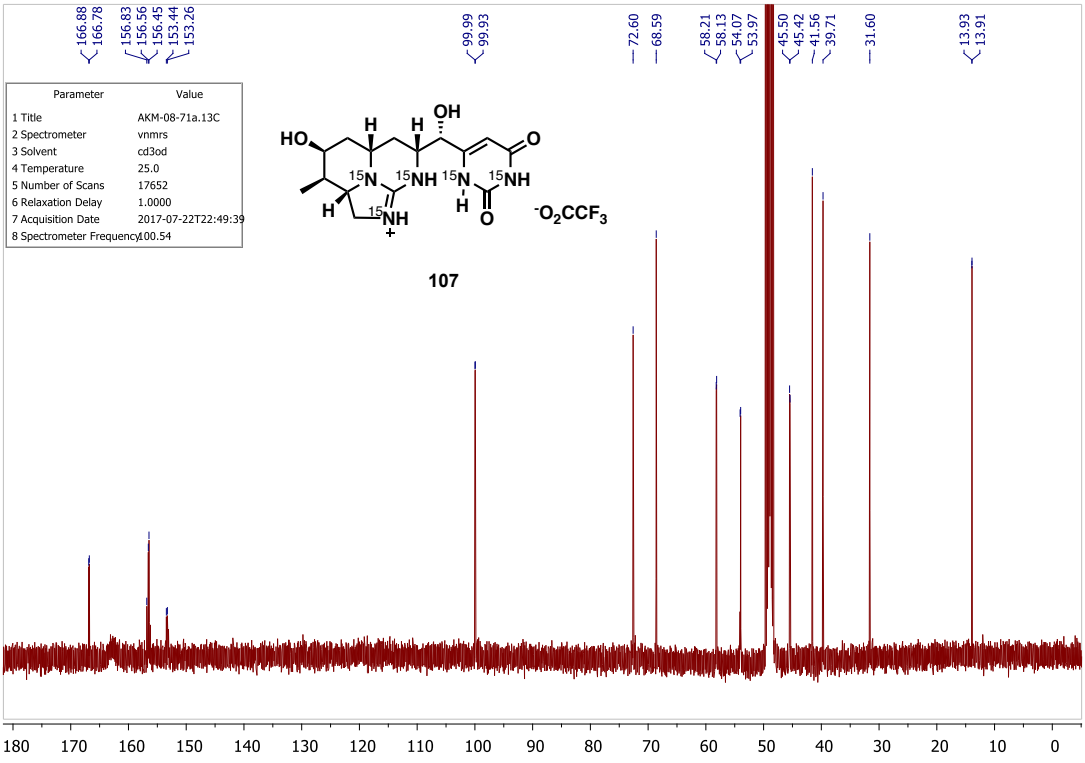

166.64 162.78 162.43 154.70 153.10 152.72
120.42 117.52 114.62
100.73
67.71
56.23
47.37 47.13 43.78 39.21 37.11 36.69 32.89
12.60
Parameter Value
1 Title AKM-07-248.13C
2 Spectrometer vnmrs
3 Solvent d2o
4 Temperature 25.0
5 Number of Scans 1092
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-04-27T00:34:18
8 Spectrometer Frequency 100.54
HO
H
H
NH
N
H
NH
O
O
H
NH
+
$^{-}O_2CCF_3$
103
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

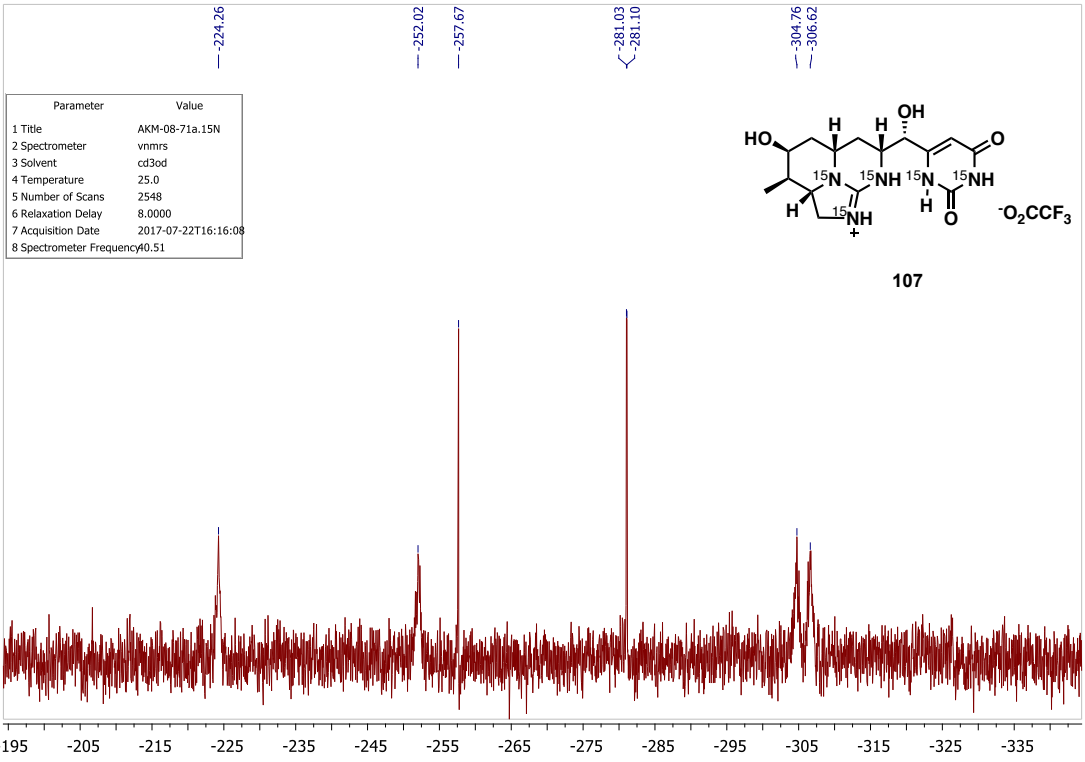

Parameter Value
1 Title AKM-08-71b_CD3OD
2 Spectrometer inova
3 Solvent cd3od
4 Temperature 25.0
5 Number of Scans 8
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-07-22T11:40:27
8 Spectrometer Frequency 599.64
106

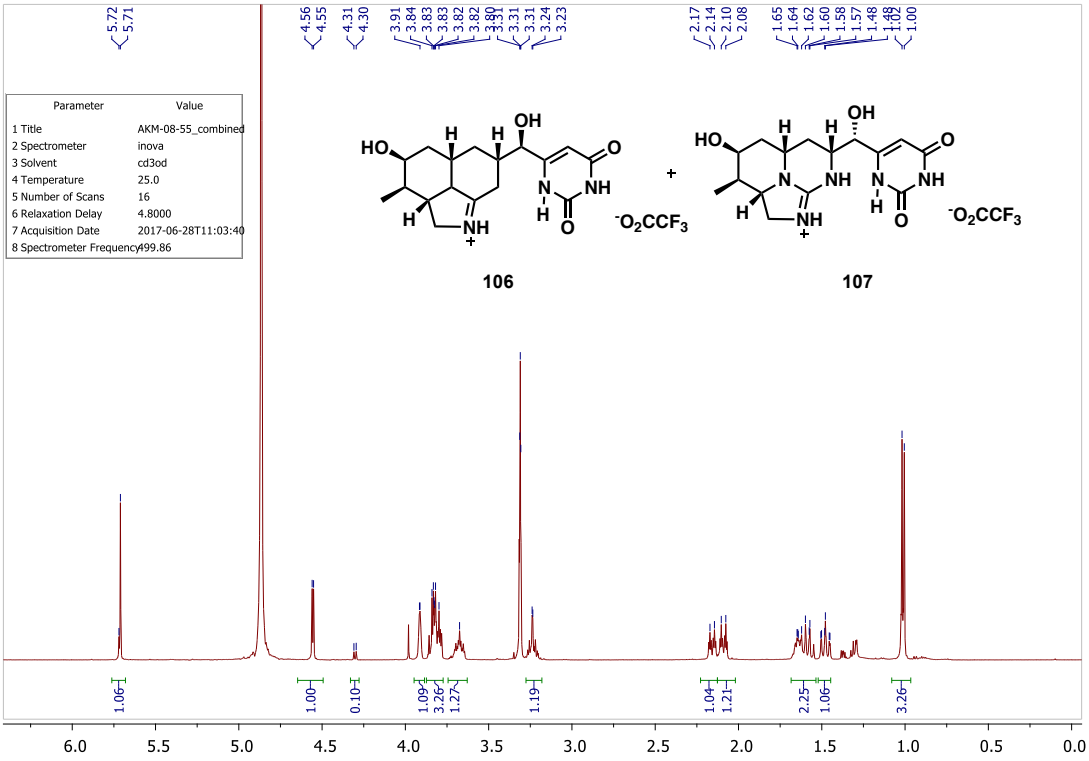

Parameter Value
1 Title AKM-08-71b.13C
2 Spectrometer inova
3 Solvent cd3od
4 Temperature 25.0
5 Number of Scans 16652
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-07-22T23:33:55
8 Spectrometer Frequency 150.79
106

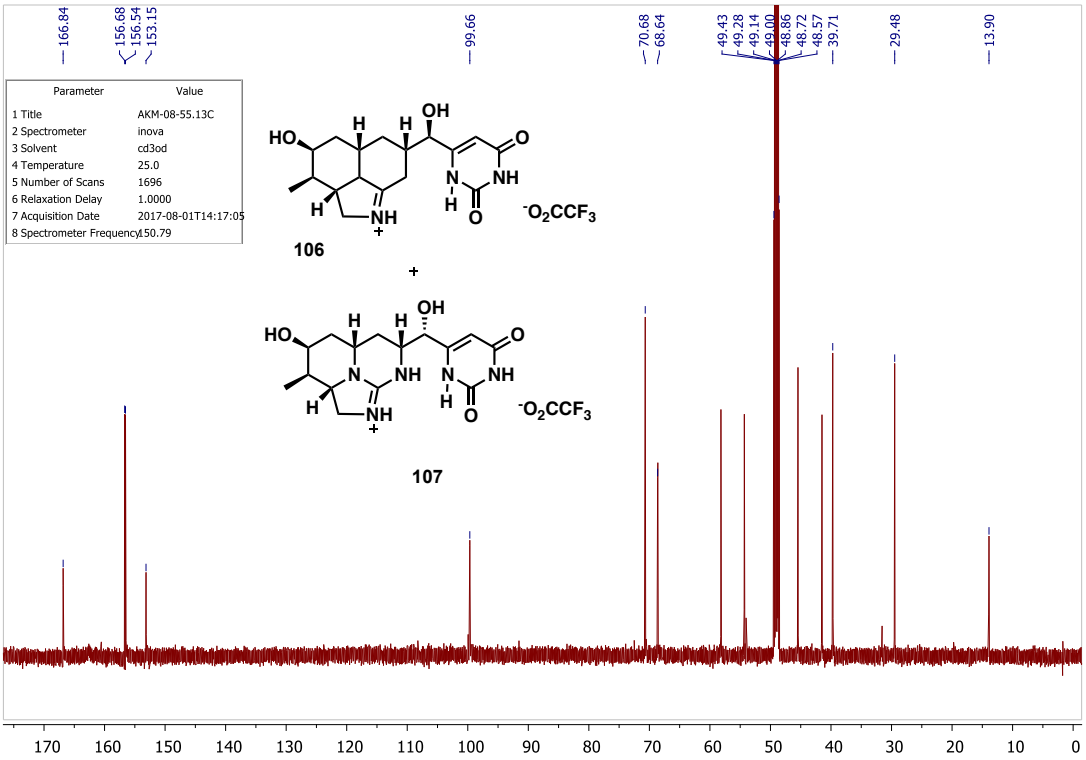

-224.43
-251.43
-280.90
-280.97
-303.81
-306.59
Parameter Value
1 Title AKM-08-71b.15N
2 Spectrometer vnmrs
3 Solvent cd3od
4 Temperature 25.0
5 Number of Scans 1000
6 Relaxation Delay 8.0000
7 Acquisition Date 2017-07-22T14:32:51
8 Spectrometer Frequency 40.51
106
-205 -215 -225 -235 -245 -255 -265 -275 -285 -295 -305 -315 -325

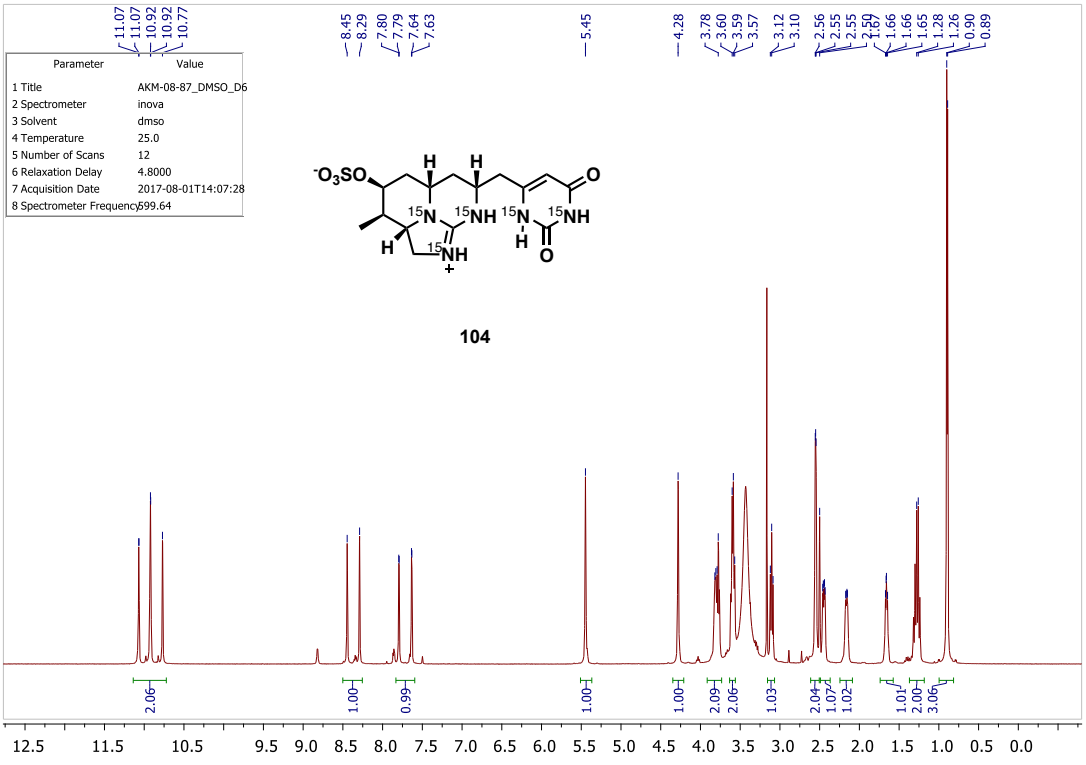

5.72 5.71 4.86 4.31 4.30 3.92 3.84 3.82 3.71 3.31 3.26 3.24 3.23 2.12 2.11 2.10 2.09 2.08 1.64 1.62 1.60 1.58 1.47 1.02 1.01
Parameter Value
1 Title AKM-08-71a_CD3OD
2 Spectrometer inova
3 Solvent cd3od
4 Temperature 25.0
5 Number of Scans 12
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-07-22T11:36:06
8 Spectrometer Frequency 599.64
107
1.03 1.00 1.01 2.04 2.07 1.00 2.00 2.08 1.00 3.04
6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

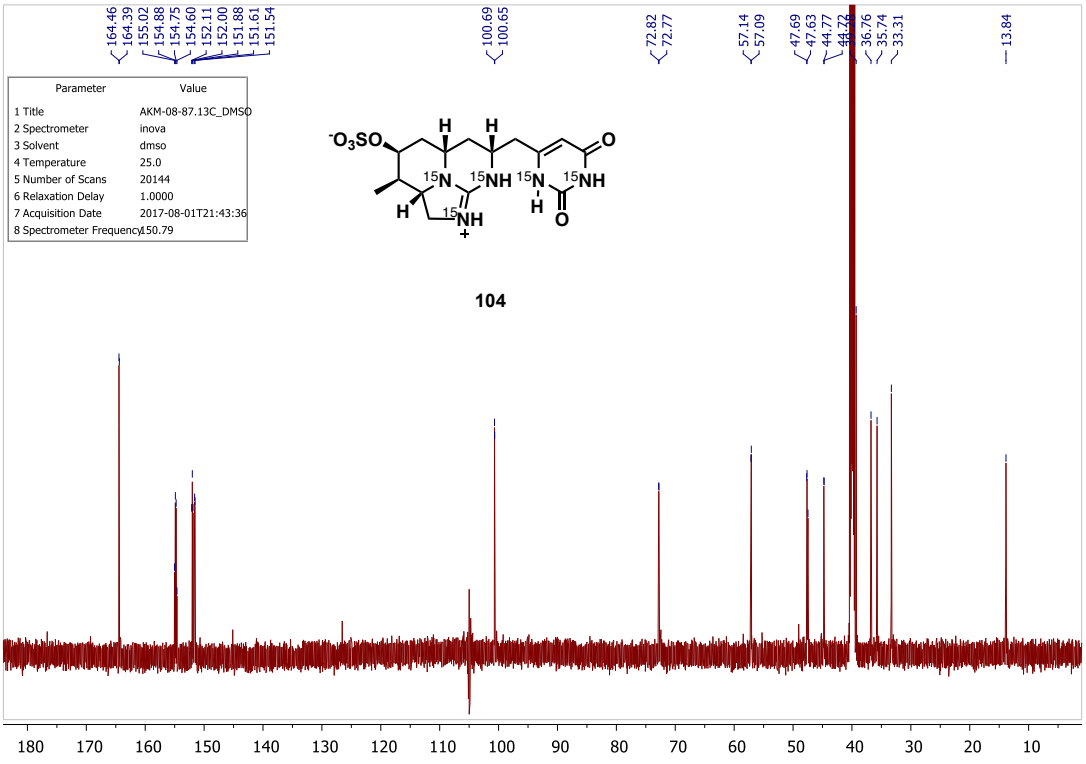

166.88
166.78
156.83
156.56
156.45
153.44
153.26
99.99
99.93
72.60
68.59
58.21
58.13
54.07
53.97
45.50
45.42
41.56
39.71
31.60
13.93
13.91
Parameter Value
1 Title AKM-08-71a.13C
2 Spectrometer vnmrs
3 Solvent cd3od
4 Temperature 25.0
5 Number of Scans 17652
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-07-22T22:49:39
8 Spectrometer Frequency 100.54
HO
H
H
OH
O
15N
15NH
15N
15NH
H
15NH
H
O
-O2CCF3
107
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

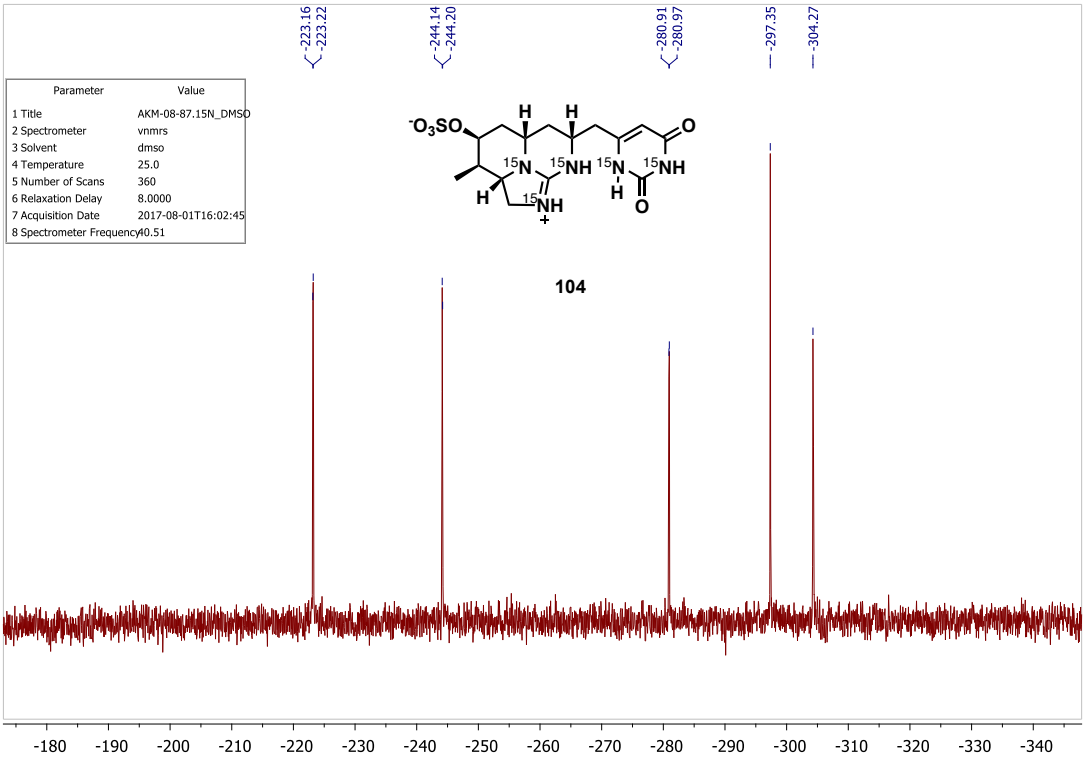

-224.26
-252.02
-257.67
-281.03
-281.10
-304.76
-306.62
Parameter Value
1 Title AKM-08-71a.15N
2 Spectrometer vnmrs
3 Solvent cd3od
4 Temperature 25.0
5 Number of Scans 2548
6 Relaxation Delay 8.0000
7 Acquisition Date 2017-07-22T16:16:08
8 Spectrometer Frequency 40.51
HO
H
H
OH
O
15N
15NH
15N
15NH
H
15NH
H
O
-O2CCF3
107
-195 -205 -215 -225 -235 -245 -255 -265 -275 -285 -295 -305 -315 -325 -335

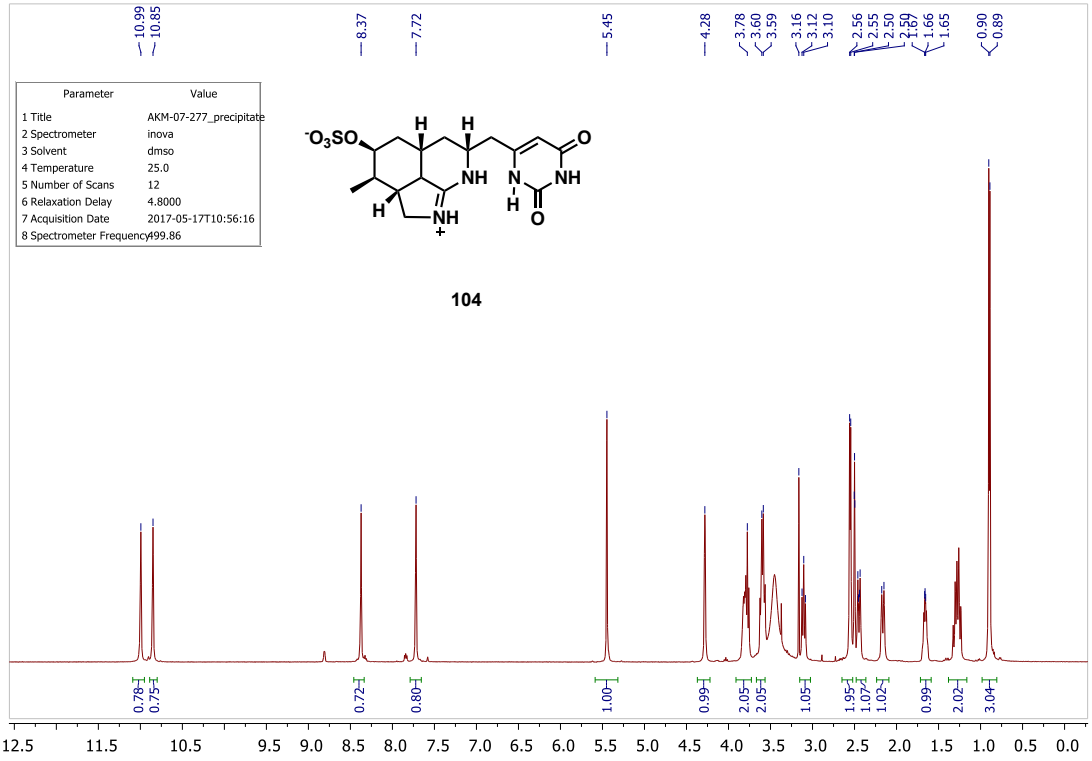

Parameter Value
1 Title AKM-08-55_combined
2 Spectrometer inova
3 Solvent cd3od
4 Temperature 25.0
5 Number of Scans 16
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-06-28T11:03:40
8 Spectrometer Frequency 499.86
106
107

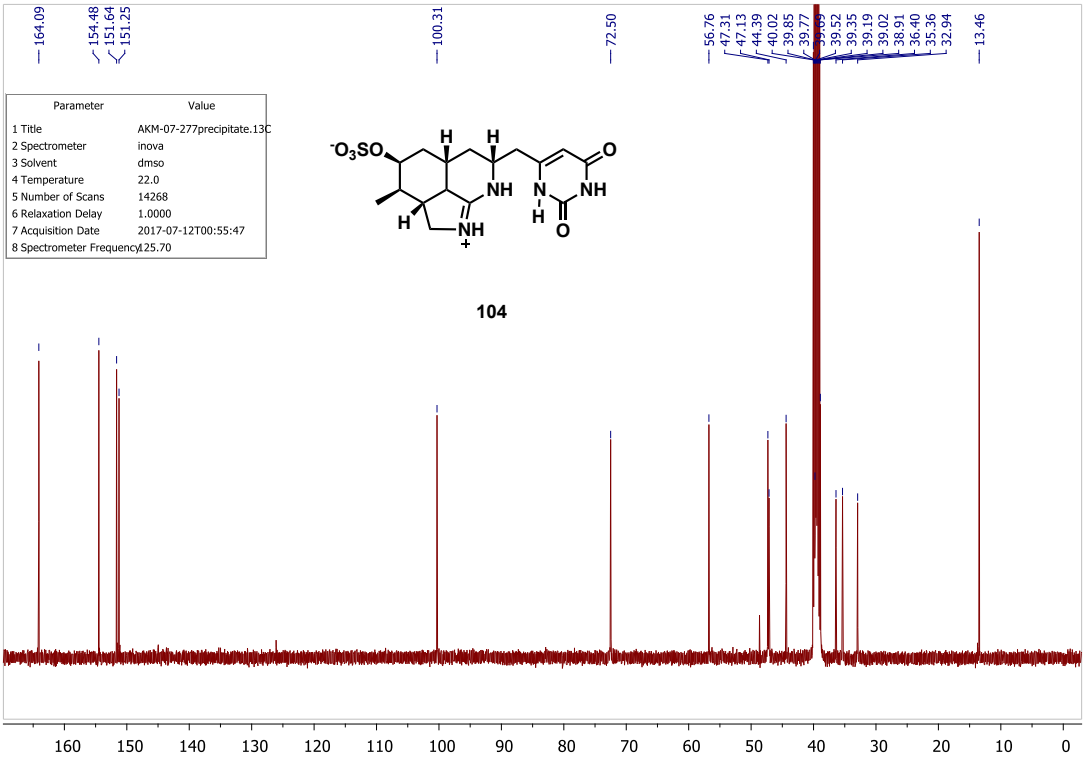

Parameter Value
1 Title AKM-08-55.13C
2 Spectrometer inova
3 Solvent cd3od
4 Temperature 25.0
5 Number of Scans 1696
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-08-01T14:17:05
8 Spectrometer Frequency 150.79
106
107

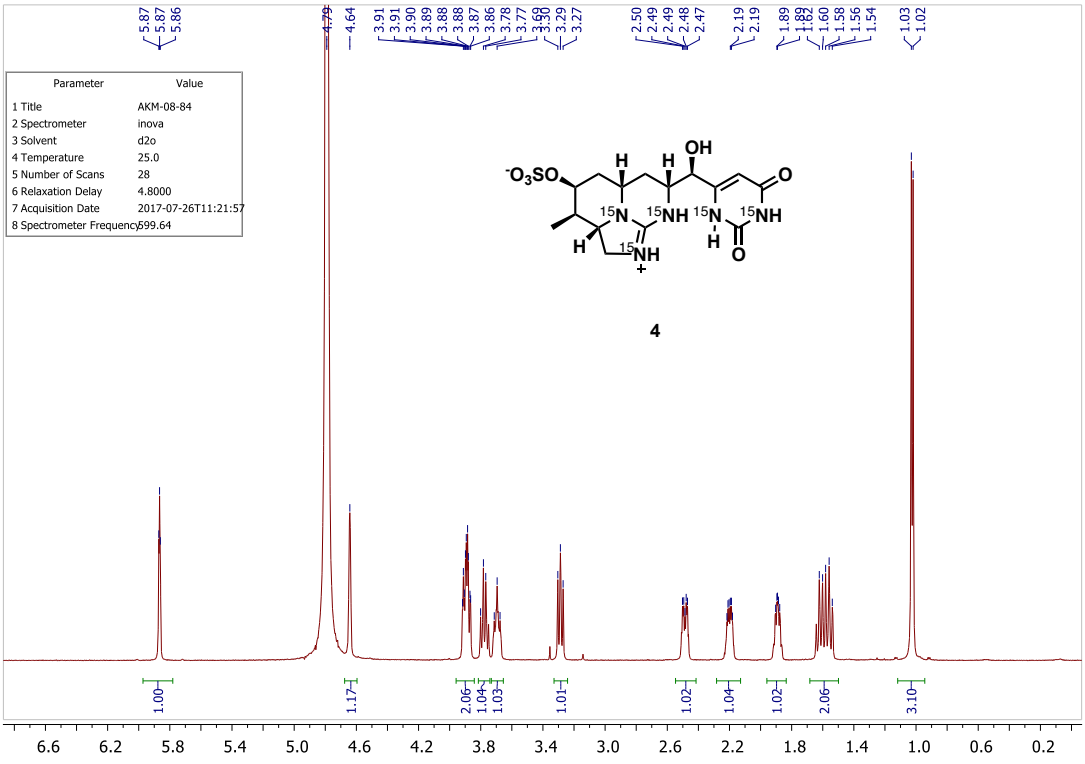

11.07
11.07
10.92
10.92
10.77
8.45
8.29
7.80
7.79
7.64
7.63
5.45
4.28
3.78
3.60
3.59
3.57
3.12
3.10
2.56
2.55
2.55
1.66
1.66
1.65
1.28
1.26
0.90
0.89
Parameter Value
1 Title AKM-08-87_DMSO_D6
2 Spectrometer inova
3 Solvent dmso
4 Temperature 25.0
5 Number of Scans 12
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-08-01T14:07:28
8 Spectrometer Frequency 599.64
104
2.06
1.00
0.99
1.00
1.00
2.09
2.06
1.03
2.04
1.07
1.02
1.01
2.00
3.06
12.5
11.5
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

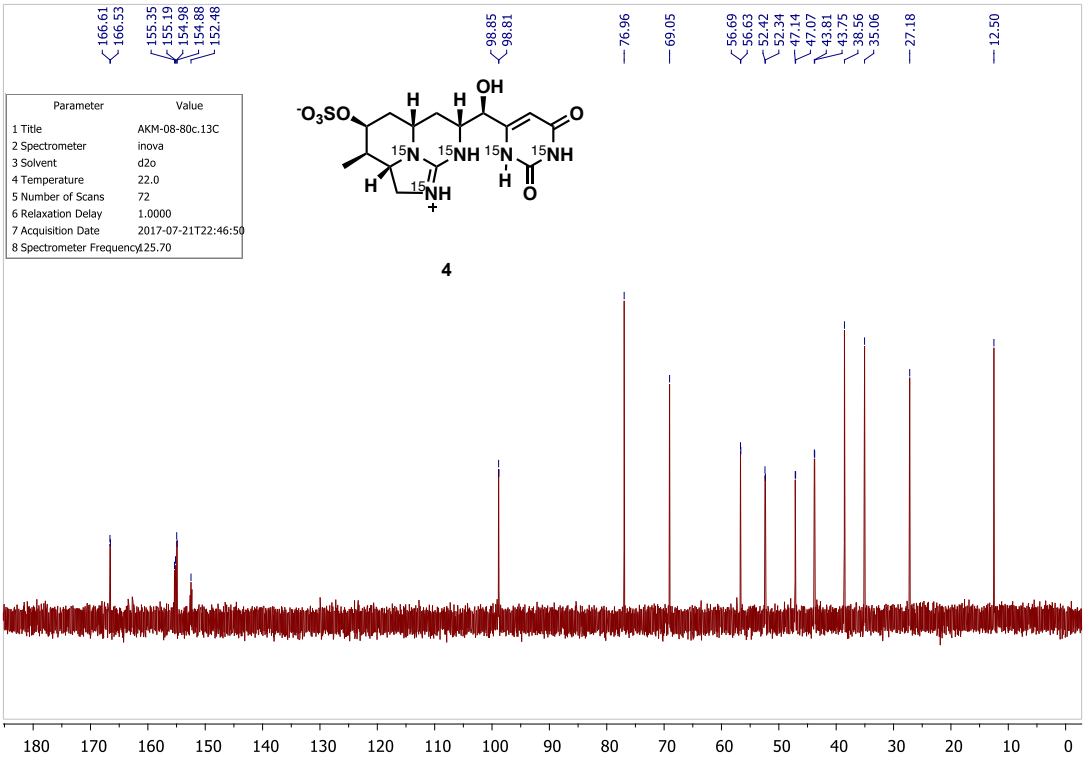

164.46
164.39
155.02
154.88
154.75
154.60
152.11
152.00
151.88
151.61
151.54
100.69
100.65
72.82
72.77
57.14
57.09
47.69
47.63
44.77
44.22
36.76
35.74
33.31
13.84
Parameter Value
1 Title AKM-08-87.13C_DMSO
2 Spectrometer inova
3 Solvent dmso
4 Temperature 25.0
5 Number of Scans 20144
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-08-01T21:43:36
8 Spectrometer Frequency 150.79
104
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

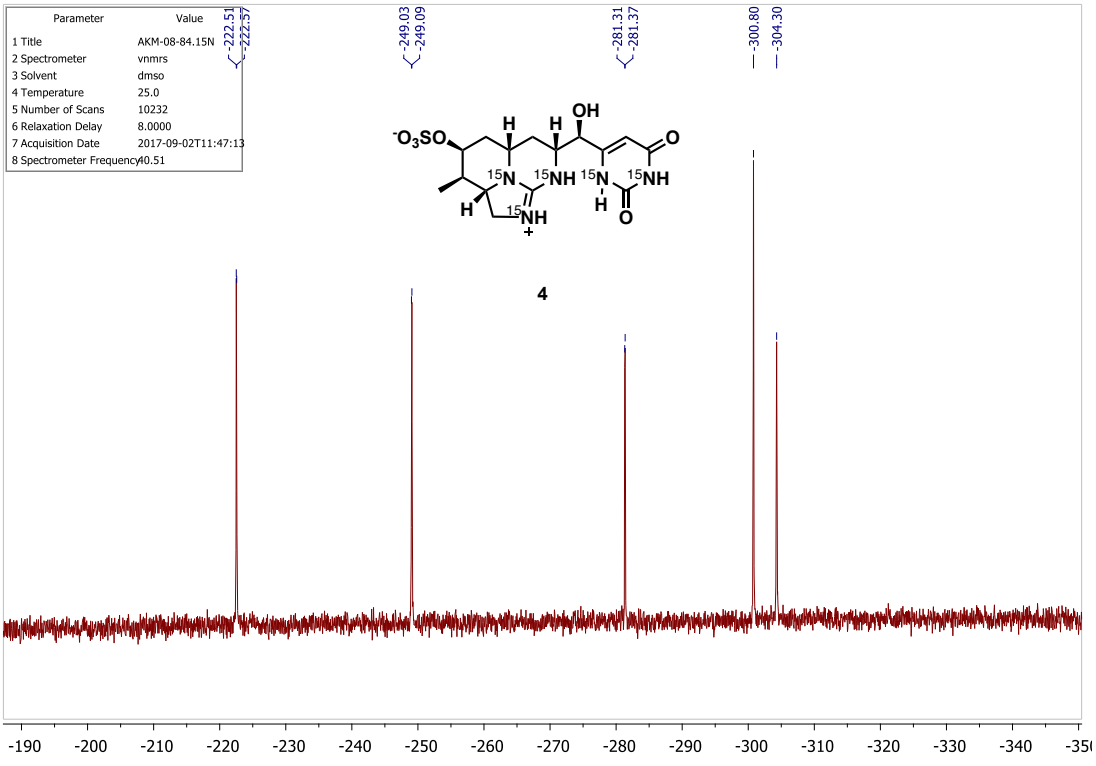

-223.16
-223.22
-244.14
-244.20
-280.91
-280.97
-297.35
-304.27
Parameter Value
1 Title AKM-08-87.15N_DMSO
2 Spectrometer vnmrs
3 Solvent dmso
4 Temperature 25.0
5 Number of Scans 360
6 Relaxation Delay 8.0000
7 Acquisition Date 2017-08-01T16:02:45
8 Spectrometer Frequency 40.51
104
-180 -190 -200 -210 -220 -230 -240 -250 -260 -270 -280 -290 -300 -310 -320 -330 -340

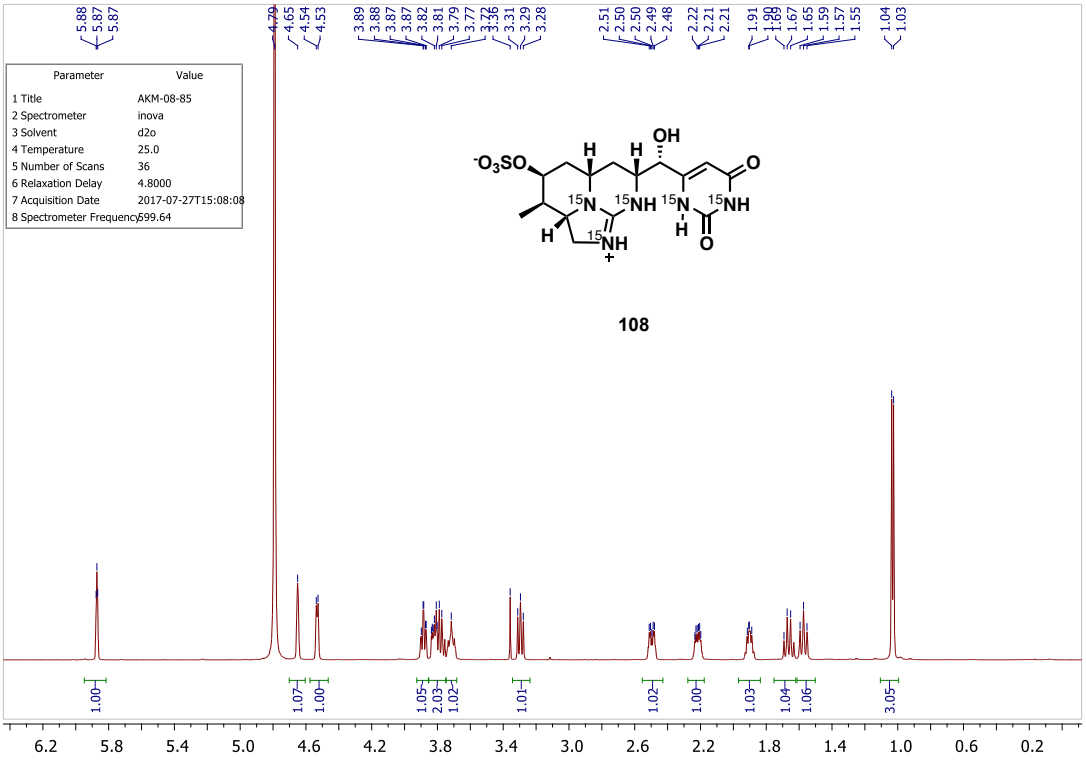

10.99
10.85
8.37
7.72
5.45
4.28
3.78
3.60
3.59
3.16
3.12
3.10
2.56
2.55
2.50
2.50
1.67
1.66
1.65
0.90
0.89
Parameter Value
1 Title AKM-07-277_precipitate
2 Spectrometer inova
3 Solvent dmso
4 Temperature 25.0
5 Number of Scans 12
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-05-17T10:56:16
8 Spectrometer Frequency 499.86
104
0.78
0.75
0.72
0.80
1.00
0.99
2.05
2.05
1.05
1.95
1.07
1.02
0.99
2.02
3.04
12.5 11.5 10.5 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

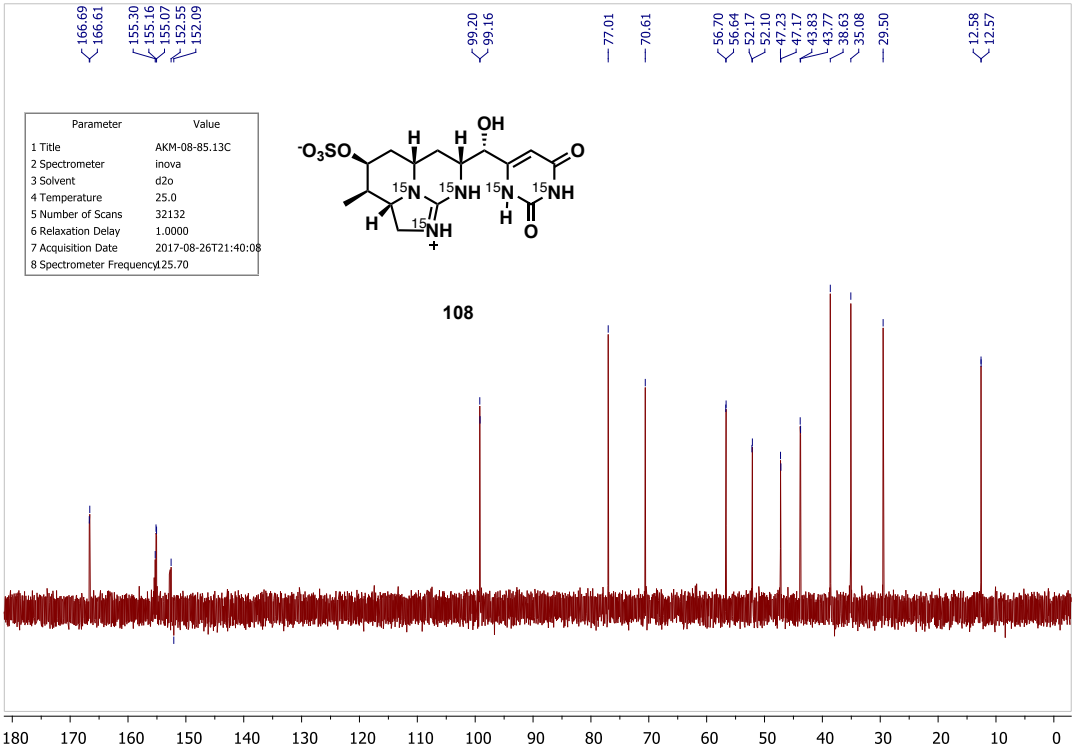

164.09
154.48
151.64
151.25
100.31
72.50
56.76
47.31
47.13
44.39
40.02
39.85
39.77
39.69
39.52
39.35
39.19
39.02
38.91
36.40
35.36
32.94
13.46
Parameter Value
1 Title AKM-07-277precipitate.13C
2 Spectrometer inova
3 Solvent dmso
4 Temperature 22.0
5 Number of Scans 14268
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-07-12T00:55:47
8 Spectrometer Frequency 125.70
104
160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

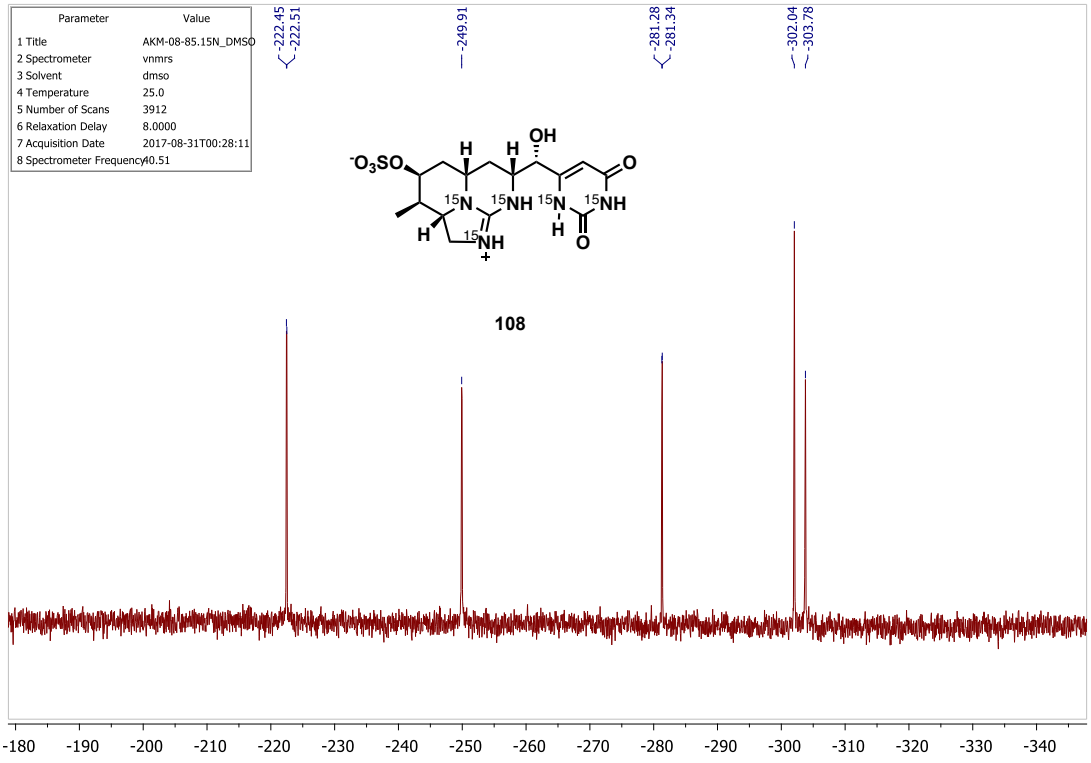

5.87
5.87
5.86
4.79
4.64
3.91
3.91
3.90
3.89
3.88
3.88
3.87
3.86
3.78
3.77
3.69
3.30
3.29
3.27
2.50
2.49
2.49
2.48
2.47
2.19
2.19
1.89
1.82
1.60
1.58
1.56
1.54
1.03
1.02
Parameter Value
1 Title AKM-08-84
2 Spectrometer inova
3 Solvent d2o
4 Temperature 25.0
5 Number of Scans 28
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-07-26T11:21:57
8 Spectrometer Frequency 599.64
4
1.00
1.17
2.06
1.04
1.03
1.01
1.02
1.04
1.02
2.06
3.10
6.6 6.2 5.8 5.4 5.0 4.6 4.2 3.8 3.4 3.0 2.6 2.2 1.8 1.4 1.0 0.6 0.2

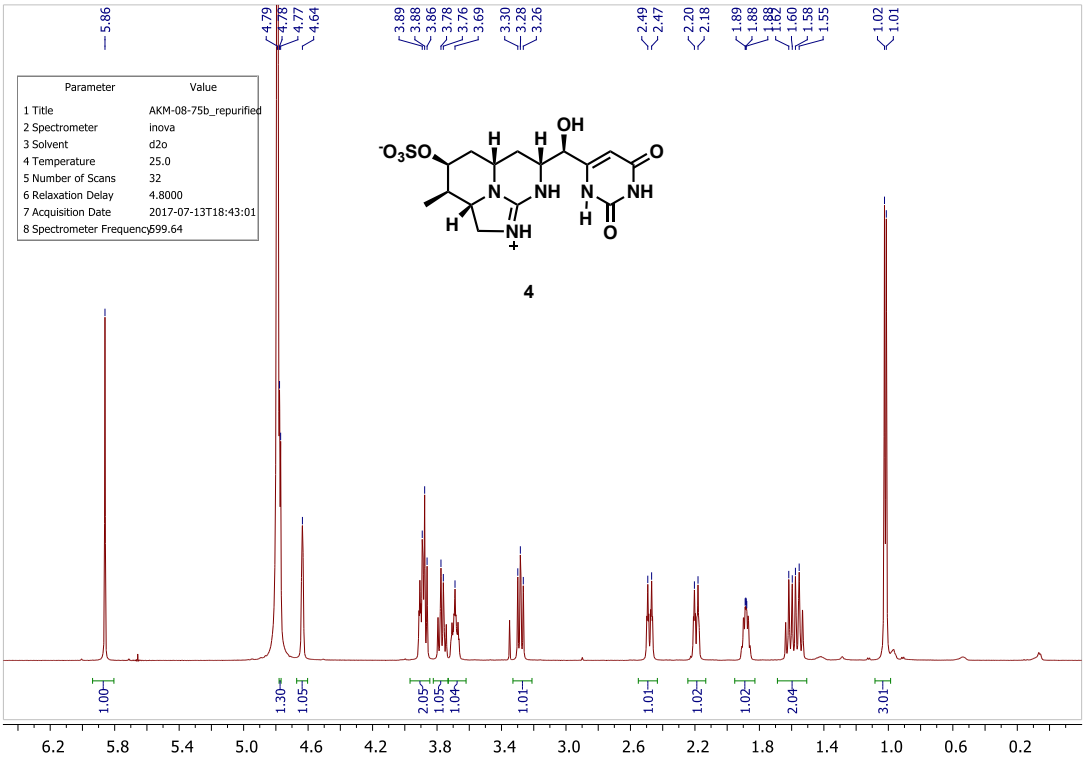

166.61
166.53
155.35
155.19
154.98
154.88
152.48
98.85
98.81
76.96
69.05
56.69
56.63
52.42
52.34
47.14
47.07
43.81
43.75
38.56
35.06
27.18
12.50
Parameter Value
1 Title AKM-08-80c.13C
2 Spectrometer inova
3 Solvent d2o
4 Temperature 22.0
5 Number of Scans 72
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-07-21T22:46:50
8 Spectrometer Frequency 125.70
4
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

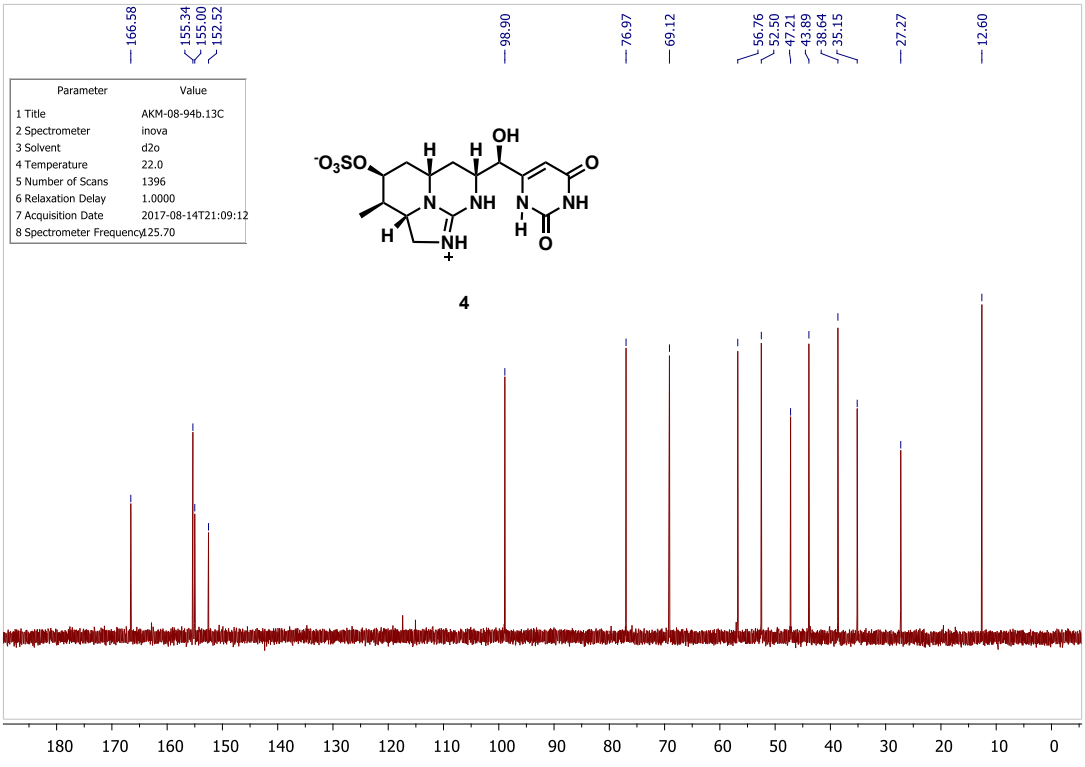

-222.51
-222.57
-249.03
-249.09
-281.31
-281.37
-300.80
-304.30
Parameter Value
1 Title AKM-08-84.15N
2 Spectrometer vnmrs
3 Solvent dmso
4 Temperature 25.0
5 Number of Scans 10232
6 Relaxation Delay 8.0000
7 Acquisition Date 2017-09-02T11:47:13
8 Spectrometer Frequency 40.51
4
-190 -200 -210 -220 -230 -240 -250 -260 -270 -280 -290 -300 -310 -320 -330 -340

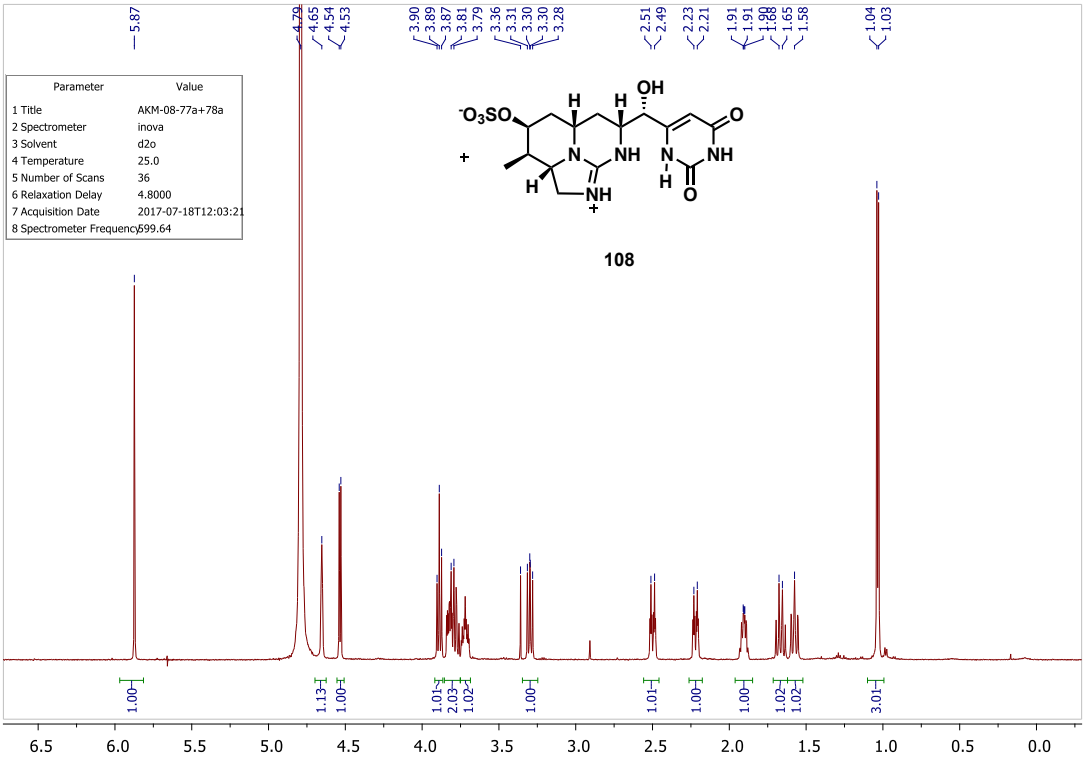

Parameter Value
1 Title AKM-08-85
2 Spectrometer inova
3 Solvent d2o
4 Temperature 25.0
5 Number of Scans 36
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-07-27T15:08:08
8 Spectrometer Frequency 599.64
108

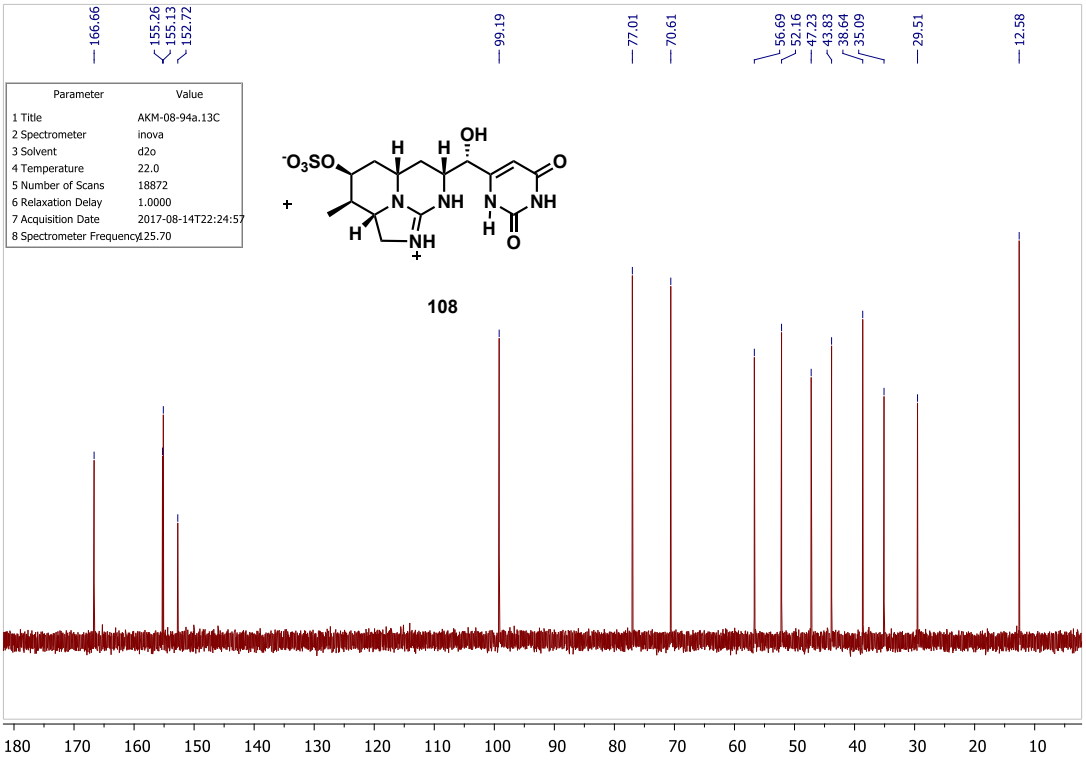

Parameter Value
1 Title AKM-08-85.13C
2 Spectrometer inova
3 Solvent d2o
4 Temperature 25.0
5 Number of Scans 32132
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-08-26T21:40:08
8 Spectrometer Frequency 125.70
108


Parameter Value
1 Title AKM-08-85.15N_DMSO
2 Spectrometer vnmrs
3 Solvent dmso
4 Temperature 25.0
5 Number of Scans 3912
6 Relaxation Delay 8.0000
7 Acquisition Date 2017-08-31T00:28:11
8 Spectrometer Frequency 40.51
-222.45
-222.51
-249.91
-281.28
-281.34
-302.04
-303.78
108
-180 -190 -200 -210 -220 -230 -240 -250 -260 -270 -280 -290 -300 -310 -320 -330 -340


5.86
4.79
4.78
4.77
4.64
3.89
3.88
3.86
3.78
3.76
3.69
3.30
3.28
3.26
2.49
2.47
2.20
2.18
1.89
1.88
1.60
1.58
1.55
1.02
1.01
Parameter Value
1 Title AKM-08-75b_repurified
2 Spectrometer inova
3 Solvent d2o
4 Temperature 25.0
5 Number of Scans 32
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-07-13T18:43:01
8 Spectrometer Frequency 599.64
4
1.00
1.30
1.05
2.05
1.05
1.04
1.01
1.01
1.02
1.02
2.04
3.01
6.2 5.8 5.4 5.0 4.6 4.2 3.8 3.4 3.0 2.6 2.2 1.8 1.4 1.0 0.6 0.2


166.58
155.34
155.00
152.52
98.90
76.97
69.12
56.76
52.50
47.21
43.89
38.64
35.15
27.27
12.60
Parameter Value
1 Title AKM-08-94b.13C
2 Spectrometer inova
3 Solvent d2o
4 Temperature 22.0
5 Number of Scans 1396
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-08-14T21:09:12
8 Spectrometer Frequency 125.70
4
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0


5.87
4.79
4.65
4.54
4.53
3.90
3.89
3.87
3.81
3.79
3.36
3.31
3.30
3.30
3.30
3.28
2.51
2.49
2.23
2.21
1.91
1.91
1.88
1.65
1.58
1.04
1.03
Parameter Value
1 Title AKM-08-77a+78a
2 Spectrometer inova
3 Solvent d2o
4 Temperature 25.0
5 Number of Scans 36
6 Relaxation Delay 4.8000
7 Acquisition Date 2017-07-18T12:03:21
8 Spectrometer Frequency 599.64
108
1.00 1.13 1.00 1.01 2.03 1.02 1.00 1.01 1.00 1.00 1.02 1.02 3.01
6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0


166.66
155.26
155.13
152.72
99.19
77.01
70.61
56.69
52.16
47.23
43.83
38.64
35.09
29.51
12.58
Parameter Value
1 Title AKM-08-94a.13C
2 Spectrometer inova
3 Solvent d2o
4 Temperature 22.0
5 Number of Scans 18872
6 Relaxation Delay 1.0000
7 Acquisition Date 2017-08-14T22:24:57
8 Spectrometer Frequency 125.70
108
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10

## References


1. Cruz, A. A. de la; Hiskia, A.; Kaloudis, T.; Chernoff, N.; Hill, D.; Antoniou, M. G.; He, X.; Loftin, K.; O'shea, K.; Zhao, C.; Pelaez, M.; Han, C.; Lynchf, T. J.; Dionysiou, D. D. *Environ. Sci.: Processes Impacts*, **2013**, *15*, 1979.
2. Hawkins, P. R.; Runnegar, M. T. C.; Jackson, A. R. B.; Falconer, I. R. *Appl. Environ. Microbiol.*, **1985**, *50*, 1292.
3. Ohtani, I.; Moore, R. E.; Runnegar, M. T. C. *J. Am. Chem. Soc.*, **1992**, *114*, 7941.
4. U.S. EPA. IRIS Toxicological Review of Cyanobacterial Toxins: Cylindrospermopsin (External Review Draft). U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-06/138, 2006; http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=160547
5. Heintzelman, G. R.; Fang, W.; Keen, S. P.; Wallace, G. A; Weinreb, S. M. *J. Am. Chem. Soc.* **2001**, *123*, 8851.
6. Harada, K. I.; Ohtani, I.; Iwamoto, K.; Suzuki, M.; Watanabe, M. F.; Watanabe, M.; Terao, K. *Toxicon*, **1994**, *32*, 73.
7. Froscio, S. M.; Humpage, A. R.; Burcham, P. C.; Falconer, I. R. *Environ. Toxicol.*, **2001**, *16*, 408.
8. Humpage, A. R.; Fontaine, F.; Froscio, S.; Burcham, P.; Falconer, I. R. *J. Toxicol. Environ. Health*, *Part A*, **2005**, *68*, 739.
9. Froscio, S. M.; Humpage, A. R.; Wickramasinghe, W.; Shaw, G.; Falconer, I. R. *Toxicon*, **2008**, *51*, 191.
10. Shaw, G. R.; Seawright, A. A.; Moore, M. A.; Lam. P. K. S. *Ther. Drug Monit.*, **2000**, *22*, 89.
11. Humpage, A. R.; Fenech, M.; Thomas, P.; Falconer. I. R. *Mutation Research/DNA Repair*, **2000**, *472*, 155.
12. Shen, X.Y.; Lam, P. K. S.; Shaw, G. R.; Wickramasinghe, W. *Toxicon*, **2002**, *40*, 1499.
13. Bain, P.; Shaw, G.; Patel, B. *J. Toxicol. Environ. Health Part A*, **2007**, *70*, 1687.
14. Chiswell, R. K.; Shaw, G. R.; Eaglesham, G.; Smith, M. J.; Norris, R. L.; Seawright, A. A.; Moore, M. R. *Environ. Toxicol.,* **1999**, *14*, 155.
15. Looper, R. E.; Runnegar, M. T. C.; Williams, R. M. *Angew. Chem., Int. Ed.,* **2005**, *44*, 3879.
16. Neumann, C; Bain, P.; Shaw, G. *J. Toxicol. Environ. Health, Part A*, **2007**, *70*, 1679.
17. Williams, C. D.; Aubel, M. T.; Chapman, A. D.; D'Aiuto, P. E.; *Lake Reservoir Manage.*, **2007**, *23*, 144.
18. Fuentes, M. S.; Rick, J. J.; Hasenstein, K. H. *J. Great Lakes Res.*, **2010**, *36*, 458.
19. Hong, Y.; Steinman, A.; Biddanda, B.; Rediske, R.; Fahnenstiel, G. *J. Great Lakes Res.*, **2006**, *32*, 645.
20. G. L. Boyer, in *Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs. Adv. Exp. Med. Biol.*, 619, ed. H. K. Hudnell, Springer Press, New York, **2008**, 153-166.
21. Eaglesham, G.K.; Norris, R. L.; Shaw, G. R.; Smith, M. J.; Chiswell, R. K.; Davis, B. C.; Neville, G. R.; Seawright, A. A.; Moore, M. R. *Environ. Toxicol.*, **1999**, *14*, 151.
22. Vogl, J.; Pritzkow, W. *Mapan J. Metrol. Soc. I.*, **2010**, *25*, 135.

23. Kubo, T.; Sano, T.; Hosoya, K.; Tanaka, N.; Kaya, K. *Toxion*, **2005**, *46*, 104.
24. Humpage, A. R.; Fenech, M.; Thomas, P.; Falconer, I. R. *Mutat. Res.*, **2000**, *472*, 155.
25. Maul, R. Enhanced LC-MS/MS analysis of cylindrospermopsin in plant and freshwater matricies using the stable isotope dilution assay. 21st Meeting of the French Society of Toxinology (SFET), December 9-10th, 2013, Pasteur Institute, Paris, France (http://sfet.asso.fr/international/program-rt/rt21-program.html)
26. Xie, C.; Runnegar, M. T. C.; Snider, B. B. *J. Am. Chem. Soc.*, **2000**, *122*, 5017.
27. Snider, B. B.; Xie, C. *Tetrahedron Lett.*, **1998**, *39*, 7021.
28. Weinreb, S. M. *Acc. Chem. Res.*, **1988**, *21*, 313.
29. Boger, D. L.; Weinreb, S. M. *Hetero Diels-Alder Methodology in Organic Synthesis*; Academic Press: San Diego, 1987; Chapter 1.
30. Weinreb, S. M. Heterodienophile Additions to Dienes. In *ComprehensiVe Organic Synthesis*; Trost, B. M., Fleming, I., Eds.; Pergamon: Oxford, 1991; Vol. 5, p 401.
31. Keen, S. P.; Weinreb, S. M. Tet*rahedron Lett.*, **2000**, *41*, 4307.
32. Heintzelman, G. R.; Fang, W.; Keen, S. P.; Wallace, G. A.; Weinreb, S. M. *J. Am. Chem. Soc.*, **2002**, *124*, 3939.
33. Comins, D. L.; LaMunyon, D. H.; Chen, X. *J. Org. Chem.*, **1997**, *62*, 8182.
34. White, J. D.; Hansen, J. D. *J. Am. Chem. Soc.,* **2002**, *124*, 4950.
35. White, J. D.; Hansen, J. D. *J. Org. Chem.,* **2005**, *70*, 1963.
36. Looper, R. E.; Williams, R. M. *Angew. Chem. Int. Ed.*, **2004**, *43*, 2930.
37. Looper, R. E.; Runnegar, M. T. C.; Williams, R. M. *Angew. Chem. Int. Ed.*, **2005**, *44*, 3879.
38. Looper, R. E.; Runnegar, M. T. C.; Williams, R. M. *Tetrahedron*, **2006**, *62*, 4549.
39. Mailyan, A. K.; Young, K.; Chen, J. L.; Reid, B. T.; Zakarian, A. *Org. Lett.*, **2016**, *18*, 5532.
40. Hart, D. J.; Djung, J. F.; Young, E. R. R. *J. Org. Chem.,* **2000**, *65*, 5668.
41. Tsuchiya, S.; Sunazuka, T.; Hirose, T.; Mori, R.; Tanaka, T.; Iwatsuki, M.; Omura, S. *Org. Lett.,* **2006**, *8*, 5577.
42. Davis, T. L. *Org. Synth.,* **1927**, *7*, 46.
43. Dukat, M.; AbdelRahman, A.; Ismaiel, A.; Ingher, S.; Teitler, M.; Gyermek, L.; Glennon, R. A. *J. Med. Chem.,* **1996**, *39*, 4017.
44. Tsubokura, K.; Iwata, T.; Taichi, M.; Kurbangalieva, A.; Fukase, K.; Nakao, Y.; Tanaka, K. *Synlett*, **2014**, *25*, 1302.
45. Li, J.; Neuville, L. *Org. Lett.,* **2013**, *15*, 6124.
46. Looper, R. E; Haussener, T. J.; Mack, J. B. C. *J. Org. Chem.,* **2011**, *76*, 6967.
47. Bernatowicz, M. S.; Wu, Y.; Matsueda, G. R. *Tetrahedron Lett.*, **1993**, *34*, 3389.
48. Drake, B.; Patek, M.; Lebl, M. *Synthesis*, **1994**, *6*, 579.
49. Katriztky, A. R.; Parris, R. L.; Allin, S. M.; Steel, P. S. *Synth. Commun.*, **1995**, *25*, 1173.
50. Poss, M. A.; Iwanowicz, E.; Reid, J. A.; Lin, J.; Gu, Z. *Tetrahedron Lett.*, **1992**, *33*, 5933.
51. Iwanowicz, E.; Poss, M. A.; Lin, J. *Synth. Commun.,* **1993**, *23*, 1443.
52. Kim, K. S.; Qian, L. *Tetrahedron Lett.,* **1993**, *34*, 7677.
53. Robinson, S.; Roskamp, E. J. *Tetrahedron*, **1997**, *53*, 6697.

54. Guo, Z.-X.; Cammidge, A. N.; Horwell, D. C. *Synth. Commun.*, **2000**, *30*, 2933.
55. Chandrakumar, N. S. *Synth. Commun.,* **1996**, *26*, 2613.
56. Yong, Y. F.; Kowalski, J. A.; Lipton, M. A. *J. Org. Chem.,* **1997**, *62*, 1540.
57. Yong, Y. F.; Kowalski, J. A.; Lipton, M. A. *Tetrahedron Lett.*, **1999**, *40*, 53.
58. Gers, T.; Kunce, D.; Markowski, P.; Izdebski, J. *Synthesis*, **2004**, *1*, 37.
59. Lammin, S. G.; Pedgrift, B. L.; Ratcliffe, A. J. *Tetrahedron Lett.*, **1996**, *37*, 6815.
60. Levallet, C.; Lerpiniere, J.; Ko, S. Y. *Tetrahedron*, **1997**, *53*, 5291.
61. Feichtinger, K.; Zapf, C.; Sings, H. L.; Goodman, M. *J. Org. Chem.*, **1998**, *63*, 3804.
62. Feichtinger, K.; Sings, H. L.; Baker, T. J.; Matthews, K.; Goodman, M. J. *J. Org. Chem.*, **1998**, *63*, 8432.
63. Streuff, J.; Hövelmann, C. H.; Nieger, M.; Muñiz, K. *J. Am. Chem. Soc.,* **2005**, *127*, 14586.
64. Bar, G. L. J.; Lloyd-Jones, G. C.; Booker-Milburn, K. I. *J. Am. Chem. Soc.*, **2005**, *127*, 7308.
65. Du, H.; Zhao, B.; Shi, Y. *J. Am. Chem. Soc.*, **2007**, *129*, 762.
66. Du, H.; Yuan, W.; Zhao, B.; Shi, Y. *J. Am. Chem. Soc.*, **2007**, *129*, 7496.
67. Xu, L.; Du, H.; Shi, Y. *J. Org. Chem.*, **2007**, *72*, 7038.
68. Muñiz, K.; Hövelmann, C. H.; Streuff, J. *J. Am. Chem. Soc*., **2008**, *130*, 763.
69. Muñiz, K.; Streuff, J.; Chavez, P.; Hövelmann, C. H. *Chem. Asian J.*, **2008**, *3*, 1248.
70. Hövelmann, C. H.; Streuff, J.; Brelot, L.; Muñiz, K. *Chem. Commun.*, **2008**, *0*, 2334.
71. Iglesias, Á.; Perez, E. G.; Muñiz, K. *Angew. Chem., Int. Ed.*, **2010**, *49*, 8109.
72. Muñiz, K.; Kirsch, J.; Chavez, P.́ *Adv. Synth. Catal.*, **2011**, *353*, 689.
73. Sibbald, P. A.; Michael, F. E. *Org. Lett.*, **2009**, *11*, 1147.
74. Chavez, P.; Kirsch, J.; Streuff, J.; Muñiz, K. *J. Org. Chem.*, **2012**, *77*, 1922.
75. Muñiz, K.; Streuff, J.; Hövelmann, C. H.; Nuńez, A. *Angew. Chem., Int. Ed.*, **2007**, *46*, 7125.
76. Zabawa, T. P.; Kasi, D.; Chemler, S. R. *J. Am. Chem. Soc.*, **2005**, *127*, 11250.
77. Zabawa, T. P.; Chemler, S. R. *Org. Lett.*, **2007**, *9*, 2035.
78. Sequeira, F. C.; Turnpenny, B. W.; Chemler, S. R. *Synfacts*, **2010**, *11*, 1291.
79. Wen, Y.; Zhao, B.; Shi, Y. *Org. Lett.*, **2009**, *11*, 2365.
80. Zhao, B.; Peng, X.; Zhu, Y.; Ramirez, T. A.; Cornwall, R. G.; Shi, Y. *J. Am. Chem. Soc.*, **2011**, *133*, 20890.
81. Wang, Y.-F.; Zhu, X.; Chiba, S. *J. Am. Chem. Soc.*, **2012**, *134*, 3679.
82. Shen, K.; Wang, Q. *Chem. Sci.*, **2015**, *6*, 4279.
83. Liu, R.-H.; Wei, D.; Han, B.; Yu, W. *ACS Catal.*, **2016**, *6*, 6525.
84. Iglesias, A.; Muñiz, K. *Chem. Eur. J.,* **2009**, *15*, 10563.
85. de Haro, T.; Nevado, C. *Angew. Chem., Int. Ed.*, **2011**, *50*, 906.
86. Muñiz, K.; Hövelmann, C. H.; Campos-Gomez, E.; Barluenga, J.; Gonzalez, J. M.; Streuff, J.; Nieger, M. *Chem. Asian J.*, **2008**, *3*, 776.
87. Li, H.; Widenhoefer, R. A. *Tetrahedron*, **2010**, *66*, 4827.
88. Chavez, P.; Kirsch, J.; Hövelmann, C. H.; Streuff, J.; Martinez-Belmonte, M.; Escudero-Adan, E. C.; Martin, E.; Muñiz, K. *Chem. Sci.*, **2012**, *3*, 2375.
89. Röben, C.; Souto, J. A.; Escudero-Adan,́ E. C.; Muñiz, K. *Org. Lett.*, **2013**, *15*, 1008.
90. Danneman, M. W.; Hong, K. B.; Johnston, J. N. *Org. Lett.*, **2015**, *17*, 2558.

91. Mizar, P.; Laverny, A.; El-Sherbini, M.; Farid, U.; Brown, M.; Malmedy, F.; Wirth, T. *Chem. Eur. J.*, **2014**, *20*, 9910.
92. Hong, K. B.; Johnston, J. N. *Org. Lett.*, **2014**, *16*, 3804.
93. Ortiz, G. X.; Kang, B.; Wang, Q. *J. Org. Chem.*, **2014**, *79*, 571.
94. Mazmouz, R.; Chapuis-Hugon, F.; Pichon, V.; Mejean, A.; Ploux, O. *ChemBioChem*, **2011**, *12*, 858.
95. Leitch, L.C.; Davidson W.M. *Can. J. Agr. Sci.*, **1949**, *29*, 189-190.
96. Laxer, A.; Major, D. T.; Gottlieb, H.G.; Fischer, B. *J. Org. Chem.*, **2001**, *66*, 5463-5481.
97. Hammerschmidt, F.; Kvaternik, H.; Schweifer, A.; Mereiter, K.; Aignerb, R. M. *Synthesis*, **2012***, 44,* 3387.
98. Jung, M. E.; Berliner, J. A.; Angst, D.; Yue, D.; Koroniak, L.; Watson, A. D.; Li, R. *Org. Lett.* **2005**, 7, 3933-3935.
99. Kim, H.; Ho, S.; Leighton, J. L. *J. Am. Chem. Soc.*, **2011**, *133*, 6517-6520.
100. Gao, X.; Townsend, I. A.; Krische, M. J. *J. Org. Chem.* **2011**, 76, 2350-2354.